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RAYMOND FRANK CRAWFORD
1891–1955

George L. McNew and Philip J. Leyendecker

In the passing of Raymond F. Crawford, Professor and Head of the Biology Department at New Mexico College of Agriculture and Mechanic Arts, plant pathology lost a loyal promoter, inspiring teacher, and skillful practitioner of its arts. He succumbed to coronary thrombosis on September 5, 1955, while visiting the home of his younger daughter in Waco, Texas.

Professor Crawford was born at Grand Junction, Iowa, on November 21, 1891, the only child of Frank Wilder and Catherine Boos Crawford. His boyhood was spent on the family farm near Grand Junction, and his interest in agriculture drew him to Iowa State College where he was granted a B.S. degree in 1920.

His interest in plant pathology was enhanced by service in barberry and Ribes eradication while he continued his studies at Iowa State College. After serving as Assistant Professor of Plant Pathology at the University of Arkansas in 1921 and 1922, he returned to Iowa State College to finish the requirements for the degree, Master of Science, in 1923. He came to New Mexico as Assistant Professor of Biology in July 1923 and was promoted to Professor and Head of the Department the following year.

As he began his 31-year stewardship of the department, no one could have predicted the influence he was to exert on his profession. Surely there was no bright promise in a student body of only 200 to provide major students, a predominantly livestock state with only limited irrigated valleys and dry-land farms, hundreds of miles of dusty roads to navigate over desert and mountains from one farming community to another, and endless teaching assignments semester after semester.

As the years unfolded, undying optimism, incredible patience, and untiring effort paid their dividends. As he taught his favorite courses in plant pathology and bacteriology, students grasped the unending panorama of how plant diseases destroyed crops—even in arid, sunbaked New Mexico valleys—and how bacteria created epidemics in man and animals. Some 200 students majored in the department, despite Professor Crawford's insistence upon exceptionally high academic standards in all major students. At least 20 of them went on to do graduate work in plant pathology, and a surprising percentage assumed positions of responsibility and leadership. This record is amazing in view of the size of the institution.

He carefully catalogued the prevalence and destructiveness of various crop diseases in a most exemplary type of survey. His research interests were reflected in various scientific reports and press releases covering such subjects as powdery mildew of peas, the nature of apple measles, fireblight control, chlorosis of trees, Phymatotrichum root rot of cotton, and cause and control of Fusarial wilt of chile peppers. He was one of the earliest advocates of sulfuric acid delinting of cotton seed to destroy surface-borne pathogens. He demonstrated that chile wilt could be adequately controlled by reducing soil temperatures by special cultural methods and preparation of seed beds.

RAYMOND FRANK CRAWFORD
1891–1955

The necessity of regulating the shipment and use of infected plant materials soon became apparent. He organized, promulgated, and then administered New Mexico's Plant Quarantine Laws. This extra activity occupied many of his off-duty hours. His sound counsel soon came to be sought in interpreting and applying such quarantine laws. Because of this, he was a member of the Western Plant Quarantine Board for over 20 years.

Although Professor Crawford was a quiet, unassuming man, he quickly gained the respect, admiration, and loyalty of his associates and students. He gave unstintingly of his time and intellectual resources in helping students find themselves. He followed their halting footsteps with keen analytical insight and observed their growth and development with unselfish pride. It was inevitable that he would leave scores of them to mourn his passing.

He is survived by his wife, Elizabeth Remele, by two daughters, and by six grandchildren.

PHYTOPATHOLOGY for March (46: 131-186) was issued March 31, 1956

SEMIAUTOMATIC EQUIPMENT FOR INJECTING INSECTS WITH MEASURED AMOUNTS OF LIQUIDS CONTAINING VIRUSES OR TOXIC SUBSTANCES[1]

Karl Maramorosch

Experimental transmissions of plant viruses to insect vectors usually have been accomplished with glass micropipettes (1, 10, 11). After 1950, the introduction of a calibrated microsyringe for injecting measured amounts of inocula served as a more precise technique, usable for quantitative studies of the influence of dosage on the incubation of viruses in insects (4, 6). The microsyringe method, however, was cumbersome and not very accurate. This paper describes a relatively simple semiautomatic quantitative method that utilizes constant carbon dioxide anesthesia applied by means of a device developed by Williams (12). The equipment required for this new method consists of a glass micropipette, tubing to connect the pipette with a pump, a bottle of carbon dioxide connected with a Büchner funnel to serve as a chamber for anesthesia, a special insect holder, a pulsating pump to force the inoculum into the insect's body, an electric time switch to operate the pump, and a dissecting microscope.

Pipettes with fine capillary tips were prepared in the manner described by Black and Brakke (2) by stretching Pyrex glass tubing 3 mm in diameter, first over a flame of a Bunsen burner, then a second time over a microburner made from a hypodermic BD No. 18 steel needle. The finer part of the capillary was cut with sharp scissors or a safety razor blade at an angle to produce a sharp end that would penetrate an insect's abdomen easily. The points of the capillary tubes were approximately 0.08 mm in outside diameter. The length of an entire Pyrex pipette was 120–150 mm, with the fine capillary tip restricted to 5 mm or less. Longer capillaries were too flexible to stand necessary pressure on the points. The detached pipettes were filled manually by suction with a few drops of the inoculum and then connected to the pulsating pump by means of a piece of rigid rubber or polyethylene tubing. A single pipette was used to inoculate as many insects as possible. After some practice, pipettes lasted long enough to inoculate 50 or more insects.

[1] Accepted for publication October 21, 1955.

The author wishes to express appreciation to Mr. Joseph Blum of Sorvall, Inc., formerly head of the Instrument Shop of the Rockefeller Institute, who constructed the time switch, insect holder, and connection of the funnel with the dissecting microscope.

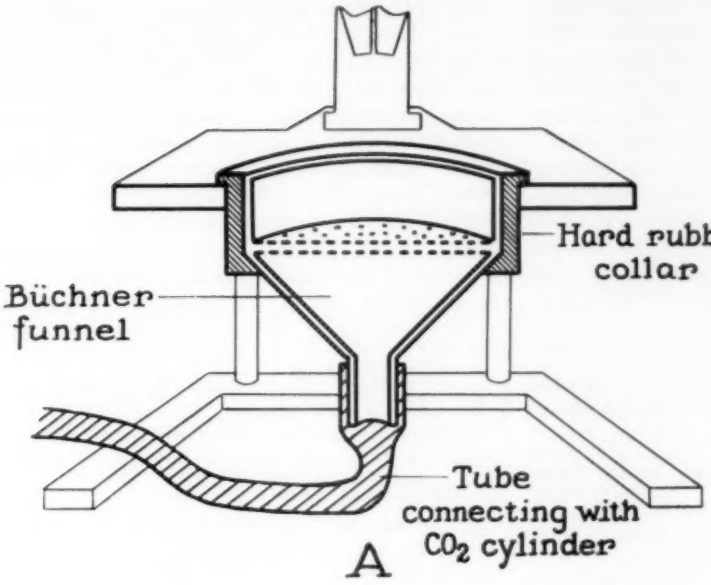
A bottle of carbon dioxide was connected with rubber tubing to a Büchner funnel, 10 cm in diameter, mounted with a hard rubber collar into the stage of a dissecting binocular microscope (Fig. 1, A).

Manipulation of anesthetized insects was by means of an insect holder (Fig. 1, B). This simple instrument permitted firm but gentle manipulation of a leafhopper without exertion of excessive pressure. The holder was constructed of 2 pieces of stainless steel wire flattened at one end to serve as forceps. These wires were fastened to a brass rod that was fitted in a metal tube that could be slid along the rod and beyond the end of the rod to hold the spring-like wires at any desired expansion.

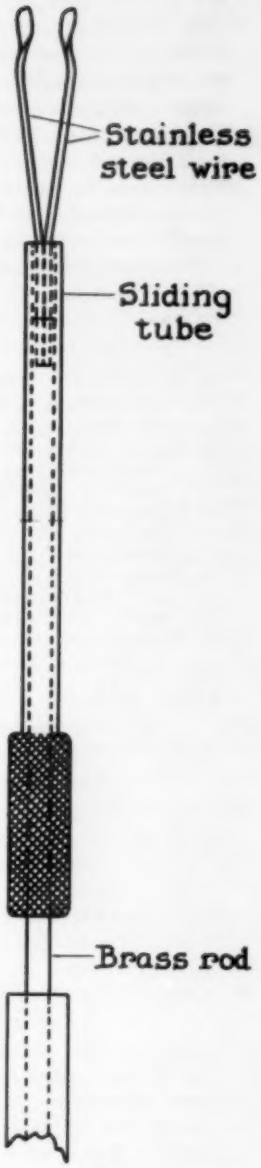
Automatic delivery of inoculum was accomplished by the use of a pulsating-type pump, as suggested by L'Heritier (3); a Thiborg aquarium aerator Model No. 1 was chosen for this purpose. The pump, connected with the Pyrex pipette, operated for periods determined by 2 adjustable cam wheels on the cam switch (Fig. 1, C). The shaft of the micromotor switch was directly connected with the cam. The speed of the motor was 10 rpm. The inoculum could be adjusted easily to the desired volume by setting the time switch. This volume depended also on the size and length of the capillary. When the time switch was adjusted properly to deliver a desired volume, this volume was repeated accurately with the same needle. In addition to uniformity in volume, the device provided also a uniform and comparatively gentle pressure during injection. It is believed that this gentle action lessened injury to the insect and favored survival.

Leafhoppers were placed in a test tube into which CO_2 was introduced. The insects were anesthetized in a matter of seconds. They were then placed on a piece of blotter paper on top of the funnel, where a continuous stream of carbon dioxide provided a bath of the anesthetic during subsequent operations. Each insect was grasped with the holder and placed in the center of the stage. The micropipette was then inserted into the abdominal cavity between the third and fourth segments and the time switch started by depressing a button. As both hands of the operator were occupied, one with the micropipette and the other with the insect holder (Fig. 1, D), the button was most conveniently depressed by foot. When the inoculum was forced into

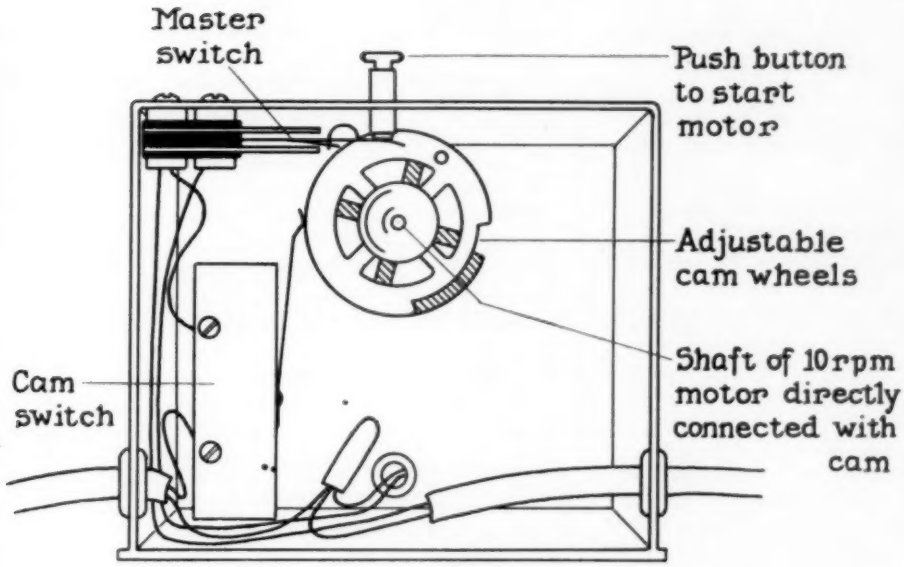
FIG. 1. Equipment for injecting insects. A) Chamber for anesthesia. B) Insect holder. C) Time switch. D) A how-to-work-the-apparatus diagram.


Hard rubber collar
Büchner funnel
Tube connecting with CO_2 cylinder

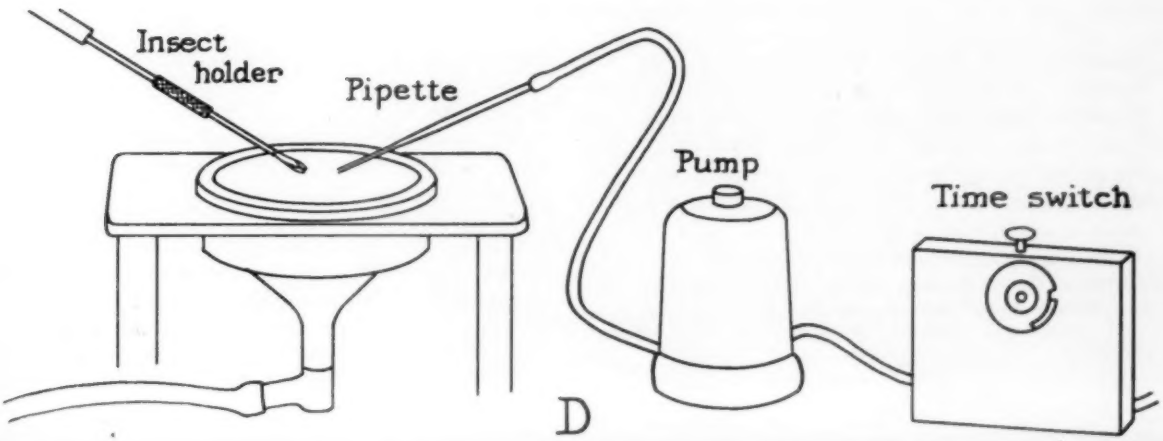
A


Stainless steel wire
Sliding tube
Brass rod

B


Master switch
Push button to start motor
Adjustable cam wheels
Shaft of 10 rpm motor directly connected with cam
Cam switch

C


Insect holder
Pipette
Pump
Time switch

D

the insect's abdomen, the leafhopper became noticeably distended. After the click of the time switch indicated the end of the operation, the needle was withdrawn carefully, and the insect was placed in a small well-aerated screened container where it soon recovered. An additional droplet was then ejected from the micropipette and removed with a piece of filter paper to avoid clogging. Mortality due to injection depends upon the skill of the operator; it usually has been very low, usually below 10 per cent.

The apparatus has been in use for more than 2 years and has proved satisfactory for leafhopper inoculations. Results from inoculations with several viruses into their respective leafhopper vectors have been published elsewhere (7, 8, 9). Although the apparatus was developed primarily for use in virus-vector work, it may find application in insect toxicology and in many other biological and chemical investigations where automatic delivery of uniform and small amounts of liquids are desired.

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NEW YORK, NEW YORK

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IDENTIFICATION OF THREE PHYSIOLOGIC RACES OF VENTURIA INAEQUALIS [1]

J. R. Shay and E. B. Williams [2]

SUMMARY

Three distinct physiologic races of *V. inaequalis* are described. Race 1 is the race commonly encountered in the U. S. and various other countries; race 2 was collected in South Dakota and successfully attacks 1) Dolgo, 2) certain segregates of the Russian variety R12740-7A, and 3) Geneva; race 3 was collected in Nova Scotia and successfully attacks Geneva. The unusual pathogenicity of Race 2 is shown to be controlled by 3 different genes in the fungus, each specific for one of the 3 differential varieties. The virulence of Race 3 on Geneva is controlled by a single gene different from that conditioning virulence on Geneva in Race 2.

Three of the apple varieties found by Shay and Hough (7) to be resistant to the apple scab pathogen (*Venturia inaequalis* (Cke.) Wint.) have since been found to be susceptible in or near the area of their origin (or introduction). Natural infections on Alexis and Dolgo, resistant varieties of *Malus baccata*, were observed by McCrory and Shay (3) in a survey of apple plantings at Brookings, South Dakota, in July, 1951. Also in 1951, C. J. Bishop of the Canadian Experimental Farms Service, Kentville, Nova Scotia, reported (in correspondence with the authors) natural infection on the variety Geneva (*M. pumila* var. *niedzwetzkyana*, open pollinated). Collections of the pathogen from these unusual infections were studied comparatively with random collections from a number of foreign countries and from Indiana from the standpoint of pathogenicity on various clones resistant to scab. Preliminary accounts have been reported (8,

[1] Accepted for publication November 7, 1955.
Journal Paper Number 898 of the Purdue University Agricultural Experiment Station, Lafayette, Indiana.
[2] Professor and Assistant Professor, respectively, of plant pathology.

9). A detailed genetic study of the strains of unusual pathogenicity was conducted simultaneously, and a preliminary report has been made (10).

MATERIALS.—*Pathogen.*—Thirty-four single-conidial isolations of *V. inaequalis* were made from infected leaves of Dolgo (including the original seedling tree), of Alexis, and of several Russian varieties at Brookings at the time of the survey of the plantings. These subsequently are referred to as the "South Dakota" collection. In the 1952 inoculations, the isolates of this collection were used singly and in groups by host variety sources. Considerable variation was noted in their behavior on Dolgo. The isolates collected from leaves of Dolgo and Alexis successfully attacked Dolgo in each case, but several of the isolates from the various Russian varieties did not. Consequently, 1 Dolgo-attacking isolate, 356-2, from Alexis was selected for most of the detailed tests.

Isolate 651 from the variety Geneva was received from J. F. Hockey, Kentville, Nova Scotia, in 1951, and others from this variety were obtained in 1953.

In addition to these collections, isolates from various commercial varieties or wild seedlings in the state of Indiana and from foreign countries were used. These are listed in Table 1.

Host.—Numerous resistant clones (7) and segregates from crosses of these clones with commercial apple varieties were inoculated in the spring of 1952 with the South Dakota isolates and with the Nova Scotia isolate (651) from the variety Geneva. It was found that these isolates behaved the same as the Indiana collection on all resistant clones except 3 varieties of *M. baccata*, certain segregates of R12740-7A, and the variety Geneva.

The Alexis, Dolgo, and Bitter Crab varieties of *M. baccata* previously were found (6) to be heterozygous for a single gene for resistance to Indiana isolates and a few northern Europe isolates (7) of *V. inaequalis*. Since all 3 varieties have appeared identical in reaction to all strains of the pathogen tested, only the data on Dolgo are presented.

R12740-7A is an apple of Russian origin that appears to carry 2 major and 3 minor genes for resistance to Indiana isolates, all in a heterozygous condition (1). In preliminary tests, 38 scab-resistant segregates from 1946-47 crosses of R12740-7A with Delicious, with Wealthy, and with Jonathan were inoculated with isolates of the South Dakota collection. Three of the 38 segregates (27-202, 44-6, and 45-39) showed greater susceptibility to the South Dakota isolates than to the Indiana isolates. From previous tests (1), it was concluded that seedlings 27-202, 44-6, and 45-39 each contained 1 gene for resistance with major effect and 2 or 3 genes with minor effects from the R12740-7A parent. Fortunately, seedling progenies of each of these 3 segregates crossed with the susceptible varieties McIntosh or Delicious were available for comparative tests with Indiana and South Dakota collections of the fungus. From these tests, seedlings were selected that showed complete susceptibility to the South Dakota collection and high resistance to the Indiana isolates. These selected seedlings were used in all subsequent comparative tests and are referred to as "backcross selections of R12740-7A". Although progeny tests of these selections must await their flowering, it is believed that they all contain the same major gene for resistance from R12740-7A and no minor genes for resistance of consequence in the host-parasite combinations studied.

The variety Geneva is resistant to Indiana isolates of *V. inaequalis* (7), but the inheritance of its resistance has not yet been determined.

TABLE 1.—*Source and number of isolates of Venturia inaequalis collected in Indiana and received from foreign countries for use in pathogenicity trials*

No. of single spore isolates	Origin	Type of apple host
38	Indiana, U.S.A.	Commercial varieties
7	Oslo, Norway	Commercial varieties
7	Finland	Commercial varieties & seedlings of *M. baccata*
2	Fjalkestad, Sweden	Unknown
2	Voldagsen uber Elze, Germany	Unknown
104	Provinces of Bosnia, Croatia, and Dalmatia, Yugoslavia	Commercial varieties & seedlings
7	Castellar, Argentina	Commercial varieties
3	Kashmir, India	Unknown
12	Ottawa, Canada	Commercial varieties

METHODS.—The methods of growing both the pathogen and the host and of carrying out the inoculations have been described previously (2, 7). For production of conidial inoculum, each isolate was cultured separately, but the water suspensions of conidia for a given group of isolates (Indiana, South Dakota, Norway, Finland, etc.) were mixed just prior to inoculation. In the case of the Yugoslav collection, however, the 104 isolates were mixed in groups of 10 or 11 each prior to inoculation.

The host reaction classes based on leaf infections are the same as those used in earlier reports, namely, 0 = no macroscopic evidence of infection; 1 = numerous or few minute pits, no sporulation; 2 = irregular or regular necrotic or chlorotic lesions, no sporulation; M = mixture of chlorotic or necrotic lesions without sporulation and lesions with restricted sporulation; 3 = restricted necrotic lesions with sporulation, no defoliation; and 4 = extensive lesions with abundant spores, infected leaves may abscise, susceptible.

All inoculations were conducted in the greenhouse from January through May of the years 1952-55.

RESULTS.—*Race differentiation.*—Clones of Dolgo, backcross selections of R12740-7A, and Geneva were forced in the greenhouse each spring of the years 1952-55. Vigorously growing trees of each of the 3 host groups were inoculated in numerous separate tests with isolates of the South Dakota collection, of the Nova Scotia collection, and of the Indiana collection. In addition, single isolates from all the collections

TABLE 2.—*Summary of inoculation tests in which 3 physiologic races of Venturia inaequalis are differentiated*

Clone	Infection class[a] incited by indicated isolates: 417-2 (Ind.) Race 1	356-2 (S.D.) Race 2	651 (Nova Scotia) Race 3
Dolgo	2	4	2
Backcross selections of R12740-7A	2	4	2
Geneva	2	2→4	4

[a] Infection classes: 2 = necrotic or chlorotic lesions, no sporulation; 4 = extensive lesions with abundant spores, susceptible; 2→4 = necrotic lesions in which fungus sporulates abundantly in moist atmosphere.

were used individually in separate tests to determine the variation in virulence among the isolates of each collection. A summary of the results of these tests is shown in Table 2. In early tests with the South Dakota isolates as a group, a resistant 2 reaction was incited on Geneva. In later tests with isolate 356-2 alone, however, it was discovered that the fungus in the necrotic lesions could be induced to sporulate if the inoculated Geneva trees were returned to the moist chamber for 24 hours at the end of a normal 14-day incubation period. This unusual reaction is indicated by the symbol 2→4 in Table 2 and subsequent tables to suggest the progression under suitable environment of the original resistant 2 reaction to the susceptible 4 type. Sporulation could not be induced in the necrotic lesions incited by the Indiana isolates by any treatment tried.

From Table 2 it is apparent that 3 distinct physiologic races can be differentiated in these collections of *V. inaequalis*. Race 1, which appears to be the common race, incites necrotic lesions, in which there is no sporulation, on all 3 host groups. Race 2 is complex in that it is able to attack successfully all 3 groups, but only if suitable environment is provided in the case of Geneva. Race 3 incites a resistant 2 reaction on Dolgo and the backcross selections of R12740-7A, but a fully susceptible reaction on Geneva.

Segregation of genes for virulence.—It was of considerable interest and of practical importance in the scab-resistance breeding program to know the number of different genes in the pathogen conditioning the unusual reactions of Races 2 and 3. To this end, isolates 651 (Race 3) and 356-2 (Race 2) were mated in culture in 1953, and a number of ascospore progenies were isolated. These were used to inoculate Dolgo, the backcross selections of R12740-7A, and Geneva. The results of tests of a few selected asci are given in Table 3. From these data, it appears that virulence on Dolgo is controlled by a single gene and that virulence on the backcross selections of R12740-7A is controlled by a different single gene. Each gene segregated in 1:1 ratio in the ascus and the 2 virulence characters are not consistently associated in the ascospore progenies. On Geneva, a 1:1 ratio occurred in Ascus 785 of the 2→4 host-parasite reaction, typical

TABLE 3.—*Segregation of virulence on differential apple varieties in ascospore progeny of Venturia inaequalis from a cross of South Dakota isolate 356-2 with Nova Scotia isolate 651*

V. inaequalis cultures	Infection class[a] incited on indicated apple variety: Dolgo	Backcross selections of R12740-7A	Geneva
Parental isolates			
356-2 (S.D.)	4	4	2→4
651 (Nova Scotia)	2	2	4
Ascus 751			
Pairs of ascospores I	2	2	4
Pairs of ascospores II	4	4	2
Pairs of ascospores III	4	4	2→4
Pairs of ascospores IV	2	2	4
Ascus 784			
Pairs of ascospores I	4	2	4
Pairs of ascospores II	4	4	2
Pairs of ascospores III	2	4	4
Pairs of ascospores IV	2	2	2→4
Ascus 785			
Pairs of ascospores I	2	2	2→4
Pairs of ascospores II	4	2	2→4
Pairs of ascospores III	2	4	4
Pairs of ascospores IV	4	4	4

[a] Infection classes: 2 = necrotic or chlorotic lesions, no sporulation; 4 = extensive lesions with abundant spores, susceptible; 2→4 = necrotic lesions in which fungus sporulates abundantly in moist atmosphere.

of culture 356-2 on Geneva, and of the 4 host-parasite reaction, characteristic of culture 651 on Geneva. In Ascus 751, and 784, however, a 1:2:1 ratio of the 2, 4, and 2→4 host-parasite reactions occurred. The resistant 2 host-parasite reaction is typical of that obtained when Race 1 cultures are inoculated on Geneva. These observations suggest that 2 different genes controlling virulence on Geneva are segregating in the cross. One is present in the South Dakota culture 356-2 and the other in the Nova Scotia culture 651.

Distribution of the 3 races.—The isolates of *V. inaequalis* from foreign countries listed in Table 1 were used in 4 separate inoculation tests on Dolgo, on the backcross selections of R12740-7A, on Geneva, and on a number of other scab-resistant selections. A summary of these tests is presented in Table 4. All of the foreign isolates gave disease reactions identical to those of the Indiana isolates of Race 1, not only on the 3 differential variety groups, but also on all other resistant clones. From these data, it appears that the genes conditioning virulence in Races 2 and 3 are not of common occurrence in isolates from the geographic areas sampled in these tests.

DISCUSSION. — Physiologic specialization of *V. inaequalis* on commercial apple varieties has been studied by a number of investigators (4, 5). Few, if any, of the commercial apple varieties are resistant to a sufficient proportion of the total U. S. or European population of biotypes of *V. inaequalis* to warrant their use as resistant parents in apple improvement. Consequently, there has been no useful purpose to be

TABLE 4.—*Infection classification of the differential varieties and other scab resistant clones following inoculations with collections of Venturia inaequalis from foreign countries*

Variety or seedling	Probable no. of major genes for resistance[a]	Infection class[b]	
		Indiana isolates	Foreign isolates[c]
Differential varieties			
Dolgo	1	2	2
Backcross selection of R12740-7A	1	2	2
Geneva	?	2	2
Other resistant clones			
Wealthy × R12740-7A			
27-27	2	1	1
27-69	1	2	2
27-202	1+	2	2
27-330	2	1	1
R12740-7A × Delicious			
45-4	1+ or 2	2	2
45-20	1	2	2
45-39	1	2	2
Jonathan × R12740-7A			
65-105	1	2	2
Golden Delicious × 26829-2-2 = Rome × *M. floribunda* 821 sib.			
14-145	1	2	2
Wolf River × *M. atrosanguinea* 804			
19-27	1	1	1
69-52	1	1	1
69-112	1	1	1
Wolf River × *M. zumi calocarpa*			
68-64	1	1	1
68-99	1	1	1
M. prunifolia 19651 × Twenty Ounce			
40-15	1	1	1
40-23	1	1	1
Wolf River × *M. micromalus*			
76-9	1 or 2	1	1
76-12	1 or 2	M	M

[a] Major genes were resolved by tests of progeny of clones listed with Indiana isolates (1, 3, 7). Presence of minor genes of considered importance is indicated by a plus sign.
[b] Infection classes: 1 = small pits; 2 = necrotic or chlorotic lesions, no sporulation; M = mixture of lesions without sporulation and lesions with restricted sporulation.
[c] Since all foreign isolates (see Table 1) incited the same reactions, the results are grouped here for brevity.

served by describing and naming the hundreds of different physiologic races that can be differentiated by these hosts.

With the discovery of field immunity to scab in numerous varieties and species of *Malus* of diverse geographic origin, and with the increased interest in the use of these as resistant parents in apple breeding, information on biotypes of *V. inaequalis* that can attack them successfully is immediately useful. To facilitate the orderly development and presentation of this information, a system for differentiating and naming races such as that used herein seems desirable.

The origin of the genes for unusual virulence described cannot be determined with certainty. Whether the genes contained in the South Dakota isolate 356-2 originated at Brookings or were imported on infected plant material from Russia during the period of Dr. Hansen's large-scale importation of plants from the various regions of that country probably can never be determined. It is of interest that the origin of the 2 apples Dolgo and R12740-7A can be traced to 2 different importations of apple seed from Russia.

Fortunately, many of the important resistant clones proved resistant to all cultures tested. In most cases their resistance came from *Malus* species (*M. floribunda, M. atrosanguinea,* etc.) introduced from Japan and northeastern China. All attempts to obtain cultures of *V. inaequalis* from Japan and Korea have been unsuccessful because of the apparent infrequent occurrence of apple scab in those countries. It is unfortunate that populations of the pathogen in eastern Russia and in China cannot be tested at this early stage of the scab-resistance apple improvement program.

The isolates tested from Australia, South America, western Europe, Scandinavia, and the Kashmir province of India resemble the isolates from Indiana and from Ottawa, Canada, in their behavior on all the resistant hosts. Apple varieties that are being developed from the *M. floribunda, M. prunifolia,* and other as yet undefiled resistance sources may have a wide range of usefulness.

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TAXONOMY, PATHOGENICITY, AND HOST-PARASITE RELATIONS OF PHOMA TRIFOLII AND PHOMA HERBARUM VAR. MEDICAGINIS [1]

N. C. Schenck and J. W. Gerdemann

SUMMARY

Phoma herbarum var. *medicaginis*, the oldest name applied to the causal organism of black stem of alfalfa, is considered the valid name. A comparative study was made between isolates of this organism and of *Phoma trifolii*, the cause of black stem of red clover. Pycnidial formation in both species was meristogenous. On potato-dextrose agar, most isolates of *P. trifolii* required light for sporulation. *P. herbarum* var. *medicaginis* sporulated uniformly under all conditions of light and darkness. *P. trifolii* was highly variable in cultural appearance, whereas *P. herbarum* var. *medicaginis* was uniform. There was no significant difference in spore size between the 2 pathogens when grown on potato-dextrose agar, on sterilized red clover or alfalfa stems, and on artificially inoculated host tissue. Although *P. herbarum* var. *medicaginis* had more 1-septate spores on potato-dextrose agar than did *P. trifolii*, both species were predominantly nonseptate on sterilized red clover or alfalfa stems. An isolate of *P. herbarum* var. *medicaginis* that produced 1-septate spores on potato-dextrose agar produced nonseptate spores on inoculated plants in the greenhouse. Spores of *P. trifolii* consistently germinated more rapidly than spores of *P. herbarum* var. *medicaginis*. The growth rate of *P. trifolii* was more rapid than that of *P. herbarum* var. *medicaginis*.

Both organisms produced the same types of root rot and damping-off lesions on seedlings of both hosts; however, all isolates were more pathogenic on the host from which they were originally isolated than on the other. There was no consistent correlation among 3 intergrading characters—pathogenicity, spore septation, and growth rate for the 2 fungi. On leaves and stems of red clover and alfalfa, all isolates produced significantly larger lesions on the host from which they were originally isolated than on the other.

Both organisms penetrated both hosts directly and through stomata. In red clover and alfalfa leaves, both pathogens were first intercellular and became intracellular in dead or dying cells. In the stem of both hosts, hyphae were intercellular and intracellular in living cells. Both species caused girdling in young stems.

The spring black stem diseases of red clover, *Trifolium pratense* L., and alfalfa, *Medicago sativa* L., are widespread and may cause considerable reductions in yield under cool, moist conditions. These diseases may affect all parts of the plant, but usually the most striking symptoms are the black elongate lesions on the stem.

The symptoms of the 2 diseases are very similar, and the pathogens causing them are difficult to differentiate. In the field, each organism is reported to be specific for its particular host, yet under greenhouse conditions the 2 fungi can be readily cross-inoculated (4). This study was undertaken to obtain a clearer understanding of the relationships of the pathogens and the 2 diseases.

TAXONOMY.—The fungus causing black stem of alfalfa has been described under several different names. The oldest name is probably *Phoma herbarum* var. *medicaginis* West. ex Rab. (1862) (7). The disease was first described in 1908 by Stewart et al (14). In 1912 Peck (6) named the pathogen *Ascochyta imperfecta*. In 1929 Rosella (9) applied the older name *A. medicaginis* Fckl. (1867) (3) and Corneli (2) in 1932 called it *Phyllosticta medicaginis* (Fckl.) Sacc. (1884) (11). The latter 2 names were shown by Jones (5) in 1918 to be synonyms of the conidial stage of *Pyrenopezzia medicaginis* Fckl. Johnson and Valleau (4) in 1933 considered *Phoma medicaginis* Malbr. & Roum. (1886) (10) to be the valid name. Toovey et al (15) in 1936 compared several isolates of this fungus from various parts of the world and decided the valid name was *A. imperfecta*. Since then most workers have accepted their conclusions.

One of the original herbarium specimens of *P. herbarum* var. *medicaginis* was examined (7). The pycnidia were erumpent, ostiolate, and black, and they averaged 232μ in diameter. The spores were obtuse at both ends, hyaline, and cylindrical. One per cent were 1-septate. The spore length was 4.3–13.6μ and averaged 6.3μ.

Although the black stem disease was not described in connection with the early names, this does not in any way invalidate them. Since *P. herbarum* var. *medicaginis* is the oldest name, and since the original herbarium specimen is indistinguishable from present isolates, this would appear to be the valid name.

The black stem disease of red clover was first described in 1929 by Valleau and Fergus (16), and the pathogen was named *Phoma trifolii* by E. M. Johnson and Valleau (4) in 1933. At first they considered the causal organisms of both alfalfa and red clover black stem to be the same. On closer examina-

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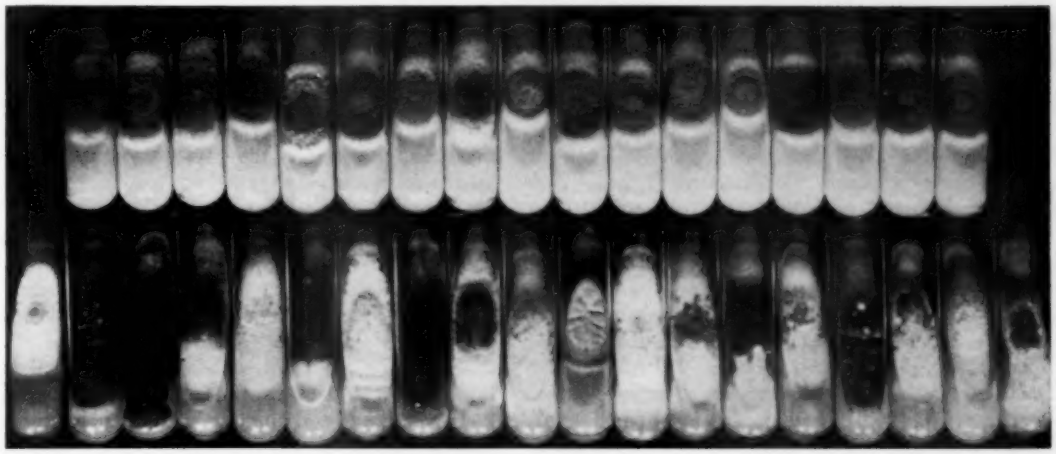

FIG. 1. Single-spore cultures after 7 days' growth on potato-dextrose agar. Top row: *P. herbarum* var. *medicaginis*. Bottom row: *P. trifolii*.

tion, they observed differences between the 2 fungi and placed them in separate species.

GENERAL MATERIALS AND METHODS.—Twenty-two isolates of *P. trifolii*, 18 collected in Illinois and 4 obtained from M. F. Kernkamp of the University of Minnesota, were compared with 18 isolates of *P. herbarum* var. *medicaginis* of which 10 were collected in Illinois, 4 were obtained from M. F. Kernkamp, and 4 were obtained from E. W. Hanson of the University of Wisconsin. All isolates were stored as single-spore cultures on potato-dextrose agar in culture-tube slants at 5°C.

Pycnidia and spores were placed for measurement in lacto-phenol containing a dye. Of the numerous dyes tested, spores of *P. herbarum* var. *medicaginis* stained best in aniline blue, whereas spores of *P. trifolii* stained best in acid fuchsin. The mean spore measurements were compared statistically according to the method of Snedecor (12), page 80.

Leaf inoculations were made in the greenhouse on potted red clover and alfalfa plants. After a thorough wetting with distilled water, the leaves were sprayed with a concentrated spore suspension. The plants were placed in a moist chamber for 4–6 days and sprayed with distilled water twice daily. Stem inoculations were made by wetting the plants in the same manner as above and then applying a spore suspension with a camel's-hair brush onto a small section of the stem, which was then wrapped with aluminum foil.

Whole leaf mounts were made by placing diseased leaves in a saturated aqueous solution of chloral hydrate containing a dye and boiling for 5 minutes. The leaves then were examined in clear lacto-phenol. For a more permanent mount, leaves were taken from chloral hydrate, rinsed in 95 per cent ethyl alcohol, in 100 per cent ethyl alcohol, and in xylene, then mounted in Permount. Other leaves were embedded in paraffin, sectioned at 12μ, and stained in safranine and fast green. Stems were examined only by means of paraffin sections.

COMPARISONS OF THE ORGANISMS.—Both pathogens developed pycnidia meristogenously in culture. The development began as 1 cell in a hypha enlarged several times the size of surrounding cells. Then it and adjacent cells divided, and the resulting hyphae entwined to form a loose ball. Mature pycnidia exuded spores in a pink- to white-colored matrix that formed a ball or loose flowing mass at the apex. Pycnidia of *P. herbarum* var. *medicaginis* were embedded in the culture medium whereas pycnidia of *P. trifolii* were raised.

Cultures of *P. herbarum* var. *medicaginis* sporulated well on potato-dextrose agar, whereas cultures of *P. trifolii* were difficult to maintain in a sporulating condition. The original cultures of *P. trifolii* sporulated well on potato-dextrose agar, but after either mass or single-spore transfer, they often sporulated sparsely.

Three replicates of each of 5 isolates from each fungus were studied for their ability to sporulate on potato-dextrose agar in total darkness, in alternating light and darkness, and in continuous light. After 14 days, *P. herbarum* var. *medicaginis* had sporulated uniformly under all light conditions. *P. trifolii* had sporulated well in continuous light, only fair in alternating light and darkness, and not at all in total darkness.

Growth characteristics of the 2 pathogens from single spores were compared on potato-dextrose agar in large culture-tubes (Fig. 1). Single-spore cultures of *P. herbarum* var. *medicaginis* were at first concentric, white, fluffy colonies that later darkened at the center and eventually became brown to black throughout. Cultures of *P. trifolii* were flat-appressed. They were faster growing and darkened more rapidly than cultures of *P. herbarum* var. *medicaginis*. Cultures of *P. trifolii* were flecked with white areas, and the edges of the colonies were irregular. Cultures of the 2 pathogens were sufficiently different at the end of 1

week to distinguish easily between them. As the cultures of each grew older, the size and appearance of the colonies became similar.

Isolates of both fungi were compared for spore size and percentage of 1-septate spores on potato-dextrose agar, autoclaved red clover stems, autoclaved alfalfa stems, and alfalfa stems sterilized with propylene oxide. After 14 days' growth at room conditions, the pycnidia were removed and examined in lacto-phenol containing a dye. Spores were measured, and the percentage of 1-septate spores was recorded.

There was no significant difference in spore size between the 2 pathogens on any of the media (Table 1). On potato-dextrose agar, spores of *P. herbarum* var. *medicaginis* were predominantly 1-septate (Fig. 2). On autoclaved red clover stems, autoclaved alfalfa stems (Fig. 3), and alfalfa stems sterilized with propylene oxide, however, isolates of this fungus produced predominantly nonseptate spores. Spores of *P. trifolii* were largely nonseptate on potato-dextrose agar (Fig. 2); however, on autoclaved alfalfa stems (Fig. 3), isolates of this fungus had a greater percentage of spore septation than did *P. herbarum* var. *medicaginis*. On sterilized red clover stems and gas-sterilized alfalfa stems, the percentage of septate spores for *P. trifolii* was about the same as for *P. herbarum* var. *medicaginis*.

Spore size and septation on potato-dextrose agar and on artificially inoculated plants were compared in tests using 1 isolate of each pathogen. One hundred spores from a suspension of each isolate were measured and the number of 1-septate spores noted. The remaining spore suspension from each isolate was used to inoculate red clover and alfalfa in the greenhouse. After 7 days in the moist chamber, typical lesions appeared on both suscepts. Several diseased leaves of red clover and of alfalfa were placed in a moist chamber in the laboratory. Sporulating pycnidia appeared 3 or 4

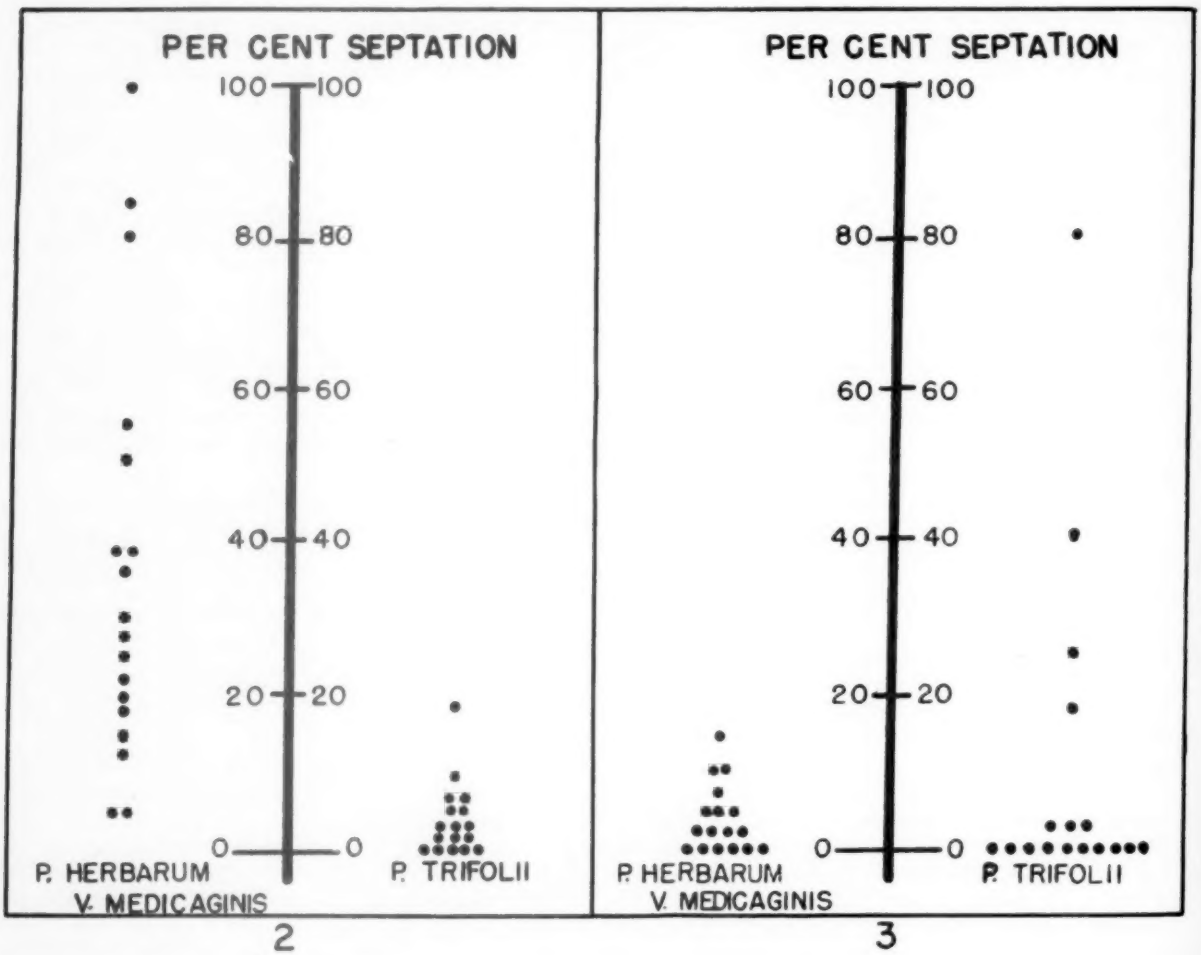

FIG. 2-3.—Fig. 2. Percentage of 1-septate spores obtained from 17 isolates of *P. trifolii* and 17 isolates of *P. herbarum* var. *medicaginis* when grown on potato-dextrose agar. Each dot represents the percentage of 1-septate spores for 1 isolate.—Fig. 3. Percentage of 1-septate spores obtained from 17 isolates of *P. trifolii* and 17 isolates of *P. herbarum* var. *medicaginis* when grown on autoclaved alfalfa stems. Each dot represents the percentage of 1-septate spores for 1 isolate.

TABLE 1.—*Spore size of P. trifolii and of P. herbarum var. medicaginis*

Media	Organism	Number of isolates[a]	Range of spore length in microns	Mean length in microns[b]
Potato-dextrose agar	*P. trifolii*	17	4.6-7.3	5.49
	P. herbarum var. *medicaginis*	17	4.5-6.3	5.12
Red clover stems (autoclaved)	*P. trifolii*	18	6.8-10.6	7.83
	P. herbarum var. *medicaginis*	16	6.8-8.7	7.60
Alfalfa stems (autoclaved)	*P. trifolii*	18	5.1-10.0	6.45
	P. herbarum var. *medicaginis*	17	5.5-7.3	6.40
Alfalfa stems (gas sterilized)	*P. trifolii*	17	5.1-10.3	6.29
	P. herbarum var. *medicaginis*	17	5.4-10.0	6.76

[a] One hundred spores measured for each isolate.
[b] Difference between means for *P. trifolii* and *P. herbarum* var. *medicaginis* not significant on the same medium.

days later.

The mean spore size of both pathogens did not differ significantly on potato-dextrose agar or living host tissue. The differences between the mean spore measurements of *P. trifolii* and *P. herbarum* var. *medicaginis* were reduced when grown on their natural host.

Thirty-eight per cent of the spores of *P. herbarum* var. *medicaginis* were 1-septate on potato-dextrose agar, but all spores produced by this isolate were nonseptate on alfalfa and red clover leaves. Spores of *P. trifolii* were nonseptate on potato-dextrose agar and on both hosts.

P. herbarum var. *medicaginis* was found sporulating abundantly on living alfalfa stems collected at Carbondale, Illinois. Pycnidia from 2 stems had all nonseptate spores, pycnidia from 6 stems had up to 10 per cent 1-septate spores, and pycnidia from 2 stems had up to 22 per cent 1-septate spores.

One culture of each pathogen was grown on potato-dextrose agar and exposed in the laboratory to direct sunlight for 4 months at room temperature. At the end of this period, 100 single spores from each culture were placed on potato-dextrose agar in Petri plates. After 14 days, several important differences were noted between the 2 isolates. Each single-spore culture of *P. herbarum* var. *medicaginis* was identical to the parent culture in appearance, whereas cultures of *P. trifolii* showed considerable variation. One variant culture of *P. trifolii* had a growth rate of half that of the 99 other cultures; 19 were white and fluffy; 45 were light brown, flat, and appressed; and 30 were dark brown to black and semiappressed.

The lack of variation in cultures of *P. herbarum* var. *medicaginis* is not in accordance with reports in the literature (1, 2, 8, 13). Possibly it may be explained by the fact that these cultures were periodically single-spored; whereas, in most reports no reference is made to single-spore cultures. Johnson and Valleau (4) stated that they single-spored both pathogens and reported variability only in *P. trifolii*.

A comparison was made between 2 isolates of each pathogen for rate of spore germination. Spores were obtained from cultures of the same age growing on the same medium. One loopful of a spore suspension was streaked onto solidified 2 per cent water agar previously adjusted to the desired temperature.

Spores of *P. trifolii* always germinated more rapidly than spores of *P. herbarum* var. *medicaginis* (Fig. 4). Spores of *P. herbarum* var. *medicaginis* did not germinate at 5° and 35°C. Spores of *P. trifolii* germinated well at 35° and germinated very slowly at 5°. After 24 hours, differences between the percentages of germinated spores of the 2 fungi became less.

The optimum temperature for growth of *P. trifolii* from mass transfer was near 20°C, whereas for *P. herbarum* var. *medicaginis* the optimum was between 20° and 25° (Fig. 5). The maximum for both pathogens was near 30°, with no growth at 35°. Isolates of *P. trifolii* grew more rapidly than isolates of *P. herbarum* var. *medicaginis* at every temperature except 5°.

Growth rates of the 2 pathogens from single spores were compared at 25°C (Fig. 6). There was no overlapping of growth curves. At the end of 6 days, the diameter of the slowest growing isolate of *P. trifolii* was 9 mm larger than the most rapid growing isolate of *P. herbarum* var. *medicaginis*. The same experiment was repeated at 22° and 27°. At these temperatures, there was some overlapping of growth curves; however, the average growth rate of the 2 species was distinctly different.

PATHOGENICITY.—All available isolates of both organisms were tested for their ability to cause damping-off. Each isolate was used to inoculate 50 seeds of alfalfa and 50 of red clover, planted in separate pots. The seeds were surface sterilized with 3.15 per cent aqueous solution of sodium hypochlorite. The inoculum was prepared by cutting the mycelial mat from 1 culture tube and fragmenting it in a Waring blender with 50 ml of distilled water. This mycelial suspension was poured directly over the seed at the time of planting. Three pots each of noninoculated red clover and alfalfa were used as controls. The total number of plants that damped-off was recorded.

Seedling emergence was recorded 7 days after planting. Analysis of variance showed no significant difference between emergence of seedlings inoculated with either pathogen, indicating that pre-emergence damping-off was the same for both organisms. The noninoculated control plants remained healthy.

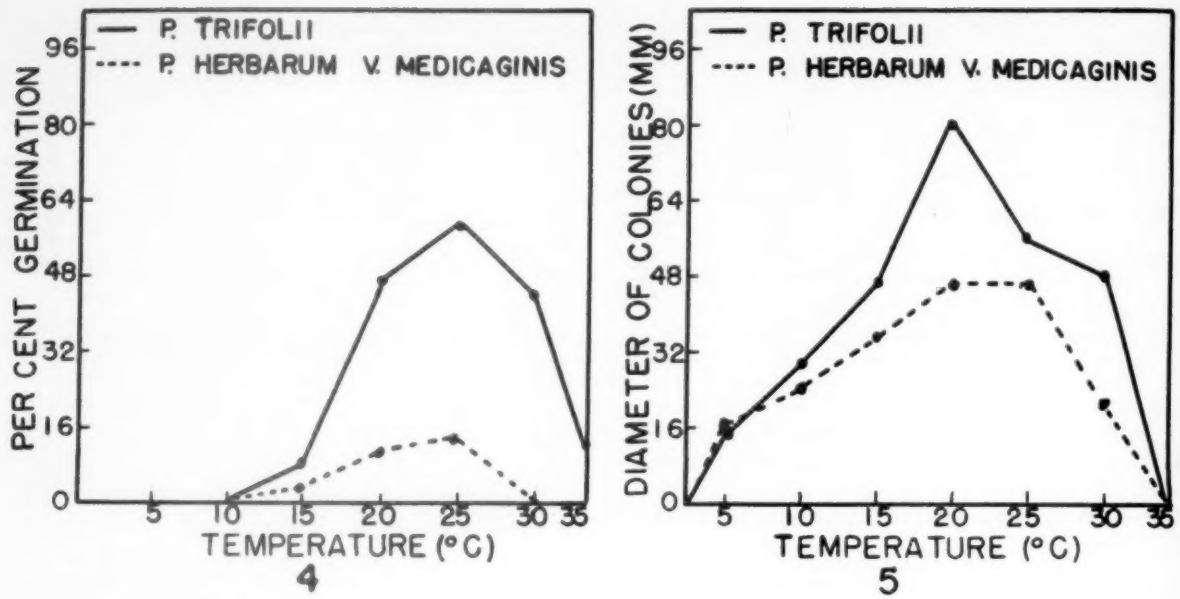

FIG. 4–5.—Fig. 4. Germination of 1 isolate each of *P. trifolii* and *P. herbarum* var. *medicaginis* after 8 hours. (Based on a count of 100 spores of each isolate at each temperature.)—Fig. 5. Average growth of 2 isolates each of *P. trifolii* and *P. herbarum* var. *medicaginis* on potato-dextrose agar at 7 different temperatures after 16 days. Colonies were from mass transfers with each isolate replicated 3 times.

An analysis of variance was calculated for the damping-off data. Of the 2 hosts used, the isolates were significantly more pathogenic on the host from which they were originally isolated (Table 2). The combined pathogenicity of *P. trifolii* was greater than the combined pathogenicity of *P. herbarum* var. *medicaginis*.

Several types of damping-off occurred. The most common type affected the seedling at, or a little above, the soil level. The tissues of the hypocotyl turned dark brown to black, the seedling was girdled at this point, and death resulted. Another type affected the cotyledons and occurred when the seed coat remained attached to them. The lesions on the cotyledons were of the typical black color associated with stem lesions of these diseases. The other type occurred when the pathogens grew into the cotyledons and killed them prior to emergence of the growing point.

TABLE 2.—*Mean number of red clover and alfalfa plants that damped-off or had root rot when seeds were inoculated with isolates of P. trifolii or of P. herbarum var. medicaginis*

Pathogen	Number of isolates	Damping-off[a]		Root rot[a]	
		Red clover	Alfalfa	Red clover	Alfalfa
Phoma trifolii	18	6.0	1.8	9.1	6.2
Phoma herbarum var. *medicaginis*	20	0.1	5.4	2.5	9.3

[a] Differences between means were significant at 1 per cent level for *P. trifolii* vs. *P. herbarum* var. *medicaginis* on alfalfa; for *P. trifolii* vs. *P. herbarum* var. *medicaginis* on red clover; and for *P. trifolii* vs. *P. herbarum* var. *medicaginis* on both hosts.

Ninety days after the start of this experiment, the roots were gently washed free of soil and examined for root lesions under 30× magnification of a binocular dissecting microscope. The number of plants with root rot was recorded and the data statistically analyzed. The results were similar to those obtained from the damping-off data (Table 2).

The most frequent type of root rot caused a brown discoloration of the outer tissues of the root slightly below soil level. Occasionally, the rot entered the xylem through a lateral root. There was no apparent difference between the type of rot produced by either pathogen.

Within species, no correlation was found between the percentage of 1-septate spores on potato-dextrose agar and pathogenicity on either host. Those isolates having the greatest number of 1-septate spores were not necessarily most pathogenic on alfalfa and least pathogenic on red clover. Nor were the least septate isolates necessarily most pathogenic on red clover and least pathogenic on alfalfa. No correlation was found within species between rate of growth and pathogenicity nor between rate of growth and spore septation. Therefore, it appears that the 2 organisms do not intergrade.

One isolate of each pathogen was used to inoculate red clover and alfalfa plants in the greenhouse, and the resulting stem and leaf lesions were tagged and measured daily. On red clover leaves, the average lesion caused by *P. trifolii* was significantly larger than the average lesion caused by *P. herbarum* var.

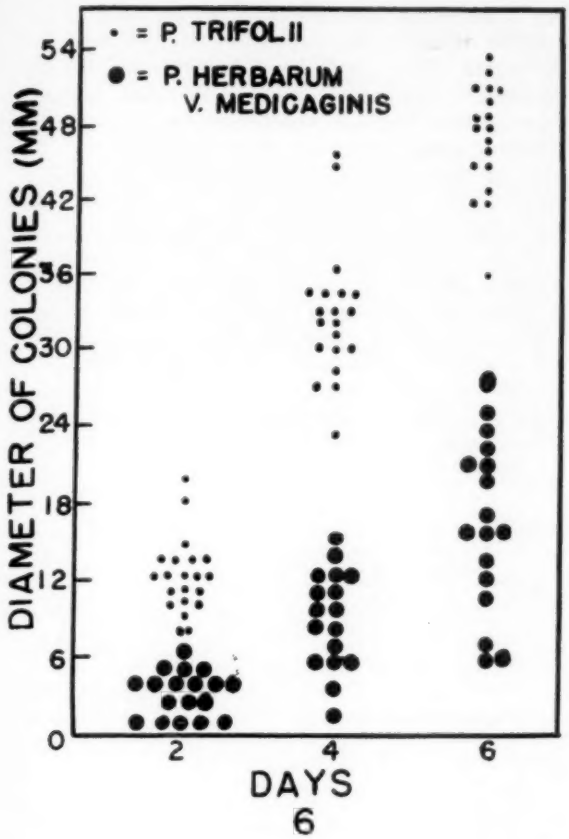

FIG. 6. Growth of 17 isolates of *P. trifolii* and of 17 isolates of *P. herbarum* var. *medicaginis* from single spores on potato-dextrose agar at 25°C. Each dot represents the average of 3 replications of 1 isolate.

medicaginis (Table 3). On alfalfa leaves, the average lesion caused by *P. herbarum* var. *medicaginis* was larger than that caused by *P. trifolii*. The leaf lesions produced by both fungi were significantly larger on red clover than on alfalfa, indicating a similarity in host response to both pathogens. *P. herbarum* var. *medicaginis* produced significantly larger lesions on alfalfa stems than did *P. trifolii*.

HOST-PARASITE RELATIONS.—Both pathogens penetrated red clover and alfalfa through stomata and directly through the cell wall. Before direct penetration, the tip of the hypha usually turned clockwise. Each pathogen penetrated without producing an appresorium. Direct penetration occurred equally well in the middle of a cell and at the juncture of 2 cells.

Following penetration, branched mycelium or a single hypha extended intercellularly 5–10 cells before injury to host cells was observed. In later stages, dead or dying cells surrounded the mycelium to a depth of several cell layers. In advanced lesions, intracellular mycelium occurred in dead, collapsed cells.

The early stages of lesion development occurred slowly, but the development was more rapid after the fungus became established. If several lesions occurred on the same leaf, they frequently coalesced and killed the leaf in 2–3 days after becoming visible. Pycnidia appeared over the surface of the leaf in 1–2 more days.

The mycelium invaded all tissues in the leaf with equal ease with the exception of the xylem, which occasionally slowed the advance of the fungus.

Both pathogens grew intercellularly and intracellularly in living cells in the stem of both hosts. Growth of the fungus usually was confined to the cortical tissues of the stem; the fungus did not enter the vascular tissue and pith until after girdling had occurred. The mycelial growth was restricted by the presence of a group of fiber cells with thick secondary walls that formed an almost continuous band outside the pholem. The fungi entered the vascular tissue and pith by growing around and between the groups of fiber cells, and hyphae were never observed in these fibers. The mycelial growth in the stems was predominantly lengthwise, resulting in the elongate appearance of black stem lesions on the stem.

The growth in young and old stems was similar; however, girdling occurred predominantly in young stems. Girdling of the cortex of old stems did not necessarily result in death. The vascular bundles were usually free of the fungus and apparently continued to function. In young stems, entrance of the fungus into the phloem and xylem enhanced the upward and downward spread of the mycelium. Growth was intracellular in dead cells of both young and old stems.

Pycnidia developed in the dead cortical tissues beneath the epidermis of the stem and ruptured the epidermis as they reached maturity. Pycnidia occurred only in extensive lesions and never developed in small lesions unless the stem was removed and placed in a moist chamber.

DISCUSSION.—The pathogens causing the spring black stem diseases of red clover and alfalfa are similar. Both had septate and nonseptate spores on all media tested, and the percentages of septate spores for both organisms overlapped. There was no statistically significant difference in spore size for the 2 pathogens on any of the media tested. Both fungi developed pycnidia meristogenously. Both penetrated the hosts in the same manner, and both produced the same type of lesions on red clover and alfalfa.

There were, however, consistent differences between the 2 fungi. Each organism was most pathogenic on

TABLE 3.—*Average size of lesions caused by P. trifolii and by P. herbarum var. medicaginis on red clover and alfalfa*

Pathogen	Leaf		Stem
	Red clover	Alfalfa	Alfalfa
	mm	mm	mm
Phoma trifolii	9.5	0.9	1.4
Phoma herbarum var. *medicaginis*	7.2	2.5	2.1

the host from which it was originally isolated. Cultural characteristics of *P. trifolii* were highly variable, whereas *P. herbarum* var. *medicaginis* was stable. Light stimulated pycnidial production in culture of *P. trifolii* but had no apparent effect on the pycnidial production of *P. herbarum* var. *medicaginis*. Spores of *P. trifolii* consistently germinated more rapidly than spores of *P. herbarum* var. *medicaginis*, and *P. trifolii* had a more rapid growth rate. The mycelium of *P. trifolii* stained well with acid fuchsin; the mycelium of *P. herbarum* var. *medicaginis* stained better with aniline blue. There was no consistent correlation between the 2 pathogens in such intergrading characteristics as growth rate, spore septation, and pathogenicity. The 2 fungi appear to be distinct but would seem to be closely related. Since the system of classification of the Fungi Imperfecti is artificial, however, it could be possible that 2 unrelated organisms might have morphological similarities. It is debatable whether the similarities in this case are sufficient to place the 2 fungi in the same species. Therefore, it seems undesirable at this time to make both organisms forms or varieties of the same species.

There can be no justification, however, for the continued use of the most recent name, *A. imperfecta*, applied to the alfalfa black stem organism. If the oldest name, *P. herbarum* var. *medicaginis*, is used, the fungus is transferred from the genus *Ascochyta* to the genus *Phoma*. Since the degree of septation in this species is highly variable, and since on host tissue the fungus is predominantly nonseptate, the genus *Phoma* is probably the more appropriate.

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PURPLE STAIN OF LEGUME SEEDS CAUSED BY CERCOSPORA SPECIES [1]

R. A. Kilpatrick and Howard W. Johnson [2]

SUMMARY

Soybean seeds, which remained fungus-free for 48 hours when plated on potato-dextrose agar containing 50 ppm of the sodium salt of 2,4-D to inhibit germination, were inoculated by placing a small agar cube containing mycelium of a Cercospora isolate on the seed coat of each seed. Of 39 isolates representing 18 *Cercospora* species from 21 different host species, 35 isolates from 20 hosts produced a pink to purple discoloration of the soybean seeds similar to that produced by *Cercospora kikuchii* under field conditions. Only *C. kikuchii* has been isolated from field-collected soybean seeds showing purple stain symptoms.

Fifteen of the isolates, 13 of which had discolored soybean seeds, were tested also on white seeds of snapbean, lima bean, lupine, and cowpea. The 13 that had discolored soybean seeds also produced a pink to purple discoloration of the seeds of these 4 other legumes.

Discoloration was evident on the seed coats within 48 hours after inoculation, and final readings were made 3–4 days later. The technique employed appears to offer a fairly rapid laboratory method for studying the discoloration of legume seeds caused by *Cercospora* spp.

The purple seed stain disease of soybeans, *Glycine max* (L.) Merrill, has been recognized in the United States for approximately 30 years. The disease is caused by the fungus (*Cercospora kikuchii*) (T. Matsu. & Tomoyasu) Gardner. The discoloration of the seed is due to the presence of the mycelium of the causal fungus in the seed coat[3]. A study of the morphology, cultural characteristics, and pathogenicity of the fungus was published by Murakishi in 1951[4]. Murakishi obtained purple discoloration of soybean seeds when he inoculated detached pods in Petri dishes in the laboratory and pods on plants in the greenhouse. Under the conditions of high relative humidity in the Petri dishes, which were kept moist with damp paper, infection of the seed occurred in nonwounded as well as in wounded pods. In the inoculations of plants in the greenhouse, wounding of the pods followed by 7 days in a moist chamber was necessary to obtain infection of the seed.

The objectives of this study were 1) to develop a more rapid method of obtaining purple discoloration of soybean seeds with *C. kikuchii;* 2) to determine if purple stain of soybean seeds could be induced by inoculation with isolates of Cercospora (other than *C. kikuchii*) from different leguminous and nonleguminous hosts; and 3) to determine if purple stain of the seeds of legumes, other than soybeans, could be obtained by inoculation with cultures of *C. kikuchii* and with Cercospora isolates from other hosts. Preliminary results have been reported earlier[5].

MATERIALS AND METHODS.—The isolates of Cercospora used in these studies included 20 from 9 leguminous hosts and 19 from 12 nonleguminous hosts. Stock cultures of all isolates were maintained on potato-dextrose agar.

The sterile soybean seeds used in the inoculations were obtained in 2 ways. 1) Seeds were removed from surface-sterilized pods under aseptic conditions and plated on potato-dextrose agar[6]. Those that had shown no fungus infection after 8 days on agar were used in the inoculations. 2) Seeds threshed out of the pods were surface sterilized in 1:1000 mercuric chloride for 1 minute, rinsed in sterile distilled water, and transferred to Petri dishes containing 25 ml of potato-dextrose agar containing 50 ppm of the sodium salt of 2,4-D (2,4-dichlorophenoxyacetic acid), which inhibited germination[7]. The seeds that showed no fungus growth after 48 hours at room temperature were transferred to other Petri dishes of potato-dextrose agar containing 2,4-D.

White seeds were used also of the following varieties of legumes: Improved Golden Wax snapbean, White Creaseback snapbean. Thorogreen lima bean, White-seeded Yellow lupine, Alabama White lupine, Lady Pea cowpea, and Early California Blackeye cowpea. These seeds were treated in the same manner as the threshed soybean seeds. In all cases, 4 fungus-free seeds were evenly spaced in a Petri dish of agar for the inoculations.

Small cubes of agar (1–2 mm) containing mycelium of the desired Cercospora isolate were cut from Petri dish cultures, and 1 cube was placed on the seed coat of each seed. At first, the inoculum was placed near the hilium of the seed, but later tests showed that

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TABLE 1.—*Discoloration of soybean seeds inoculated in Petri dishes with 39 separate Cercospora isolates from various hosts*

Cercospora species[a]	Source of isolates	Proportion of seeds discolored[b]
	(Isolates from leguminous hosts)	
C. canescens Ell. & G. Martin	*Dolichos lablab*	11/12
C. puerariicola Yam.	*Pueraria lobata*	9/12
C. kikuchii (T. Matsu. & Tomoyasu) Gardner	*Glycine max* (seed)	10/12
	G. max (seed)	3/4
	G. max (seed)	7/7
	G. max (seed)	3/4
	G. max (seed)	32/32
	G. max (funiculus)	12/12
	G. max (petiole)	38/38
	G. max (leaf)	40/40
	G. max (stem)	20/20
	G. max (stem)	20/20
	G. max (stem)	4/4
C. sojina Hara	*G. max* (leaf)	0/12
C. stizolobii H. & P. Syd.	*Stizolobium deeringianum*	12/12
C. zebrina Pass.	*Trifolium ambiguum*	11/12
	T. incarnatum	8/8
	T. subterraneum	12/12
C. cruenta Sacc.	*Vigna sinensis*	16/16
C. sp.	*Strophostyles* sp.	0/16
	(Isolates from nonleguminous hosts)	
C. brachiata Ell. & Ev.	*Amaranthus retroflexus*	16/16
C. clavata (Gerard) Cooke	*Asclepias syriaca*	16/16
C. chrysanthemi Heald & Wolf	*Chrysanthemum morifolium*	12/12
C. citrullina Cooke	*Cucurbita maxima*	12/12
C. festucae Hardison	*Festuca arundinacea*	8/8
	F. arundinacea	7/8
C. gossypina Cooke	*Gossypium hirsutum*	8/8
C. abelmoschi Ell. & Ev.	*Hibiscus esculentus*	15/15
C. penniseti Chupp	*Pennisetum glaucum*	8/8
	P. glaucum	8/8
	P. glaucum	9/12
	P. glaucum	12/12
C. sesami Zimm.	*Sesamum indicum*	9/12
C. sorghi Ell. & Ev. ?	*Sorghum halepense*	0/28
C. sorghi	*S. halepense*	8/8
	Sorghum sudanense	11/16
C. sorghi ?	*Sorghum vulgare*	0/12
C. sorghi	*S. vulgare*	9/17
	S. vulgare	11/22

[a] A question mark indicates the possibility of a different biotype.
[b] The numerator is the number of seeds discolored, and the denominator is the number inoculated.

infection would occur anywhere as long as direct contact was made between the fungus and the seed coat. A minimum of 4 seeds were inoculated with each Cercospora isolate. The Petri dishes containing inoculated seeds were kept at room temperature (70°–95°F) during the period of the tests. Observations were made at 24-hour intervals, and final readings were made 5–6 days after inoculation.

EXPERIMENTAL RESULTS.—All isolates of *C. kikuchii*, whether from soybean stems, leaves, or seeds produced a pink to purple discoloration of the inoculated soybean seeds (Table 1). Four of the isolates, all from soybean seeds, had been maintained as stock cultures for more than 3 years at the time of the inoculations. Other *Cercospora* species from leguminous hosts that discolored the soybean seeds in a similar manner were *C. canescens* from hyacinth bean; *C. puerariicola* from kudzu; *C. stizolobii* from velvet bean; *C. zebrina* from crimson clover, sub clover and *Trifolium ambiguum*; and *C. cruenta* from cowpea. Two of the Cercospora isolates from leguminous hosts did not discolor the inoculated soybean seed. These were an isolate of the soybean frogeye leafspot fungus, *C. sojina*, and an unidentified *Cercospora* species from sand bean (*Strophostyles* sp.).

Cercospora species isolated from various nonleguminous hosts also discolored the soybean seeds. These were *C. brachiata* from green pigweed; *C. clavata* from common milkweed; *C. chrysanthemi* from cultivated chrysanthemum; *C. citrullina* from summer squash; *C. festucae* from tall fescue; *C. gossypina* from upland cotton; *C. abelmoschi* from okra; *C. penniseti* from pearl millet; *C. sesami* from sesame; and *C. sorghi* from Johnson grass, Sudan grass, and sorgo.

One isolate from Johnson grass and one from sorgo did not discolor the inoculated soybean seeds, whereas

TABLE 2.—*Purple discoloration of seeds of 7 white-seeded legumes inoculated in Petri dishes with 15 separate Cercospora isolates from various hosts*

Cercospora species[b]	Proportion of discolored to inoculated seeds of 7 legumes[a]						
	Improved Golden Wax snapbeans	White Creaseback snapbean	Thorogreen lima bean	White-seeded Yellow lupine	Alabama White lupine	Lady Pea cowpea	Early Calif. Blackeye cowpea
			(Isolates from leguminous hosts)				
C. canescens	3/4		4/4	4/4			4/4
C. kikuchii	4/4	4/4	8/8	7/8	4/4	3/4	5/8
	4/4	4/4	8/8	6/8	4/4	4/4	6/8
	4/4		4/4	4/4			3/4
	3/4		4/4	3/4			3/4
	2/4		4/4	2/4			3/4
	2/4		4/4	4/4			4/4
C. stizolobii		4/4	4/4	4/4	4/4	2/4	3/4
C. zebrina	4/4		4/4	4/4			3/4
			(Isolates from nonleguminous hosts)				
C. penniseti	3/4		4/4	2/4			3/4
C. sorghi ?	0/4	0/4	0/4	0/4	0/4		0/4
C. sorghi		4/4	4/4		5/5		4/4
		8/8	4/4	4/4	4/4	2/4	3/4
C. sorghi ?		4/4	4/4	0/4	4/4	3/4	3/4
C. sorghi	4/4	4/4	4/4	9/12	8/8	1/4	3/4

[a] The numerator of the fraction is the number of seeds discolored and the denominator is the number inoculated.
[b] A question mark indicates the possibility of a different biotype.

other isolates from these hosts did cause discoloration. The isolates that did not cause discoloration were somewhat different in cultural characters from the other Sorghum isolates and may not have been *C. sorghi*, or were different biotypes of this species.

In tests with seeds of 7 varieties of white-seeded legumes, all isolates of *C. kikuchii* produced a pink to purple discoloration of the seeds of snapbean, lima bean, yellow lupine, white lupine, and cowpea (Table 2). Other *Cercospora* species from leguminous hosts that discolored the seeds of these legumes in a similar manner were *C. canescens* from hyacinth bean, *C.*

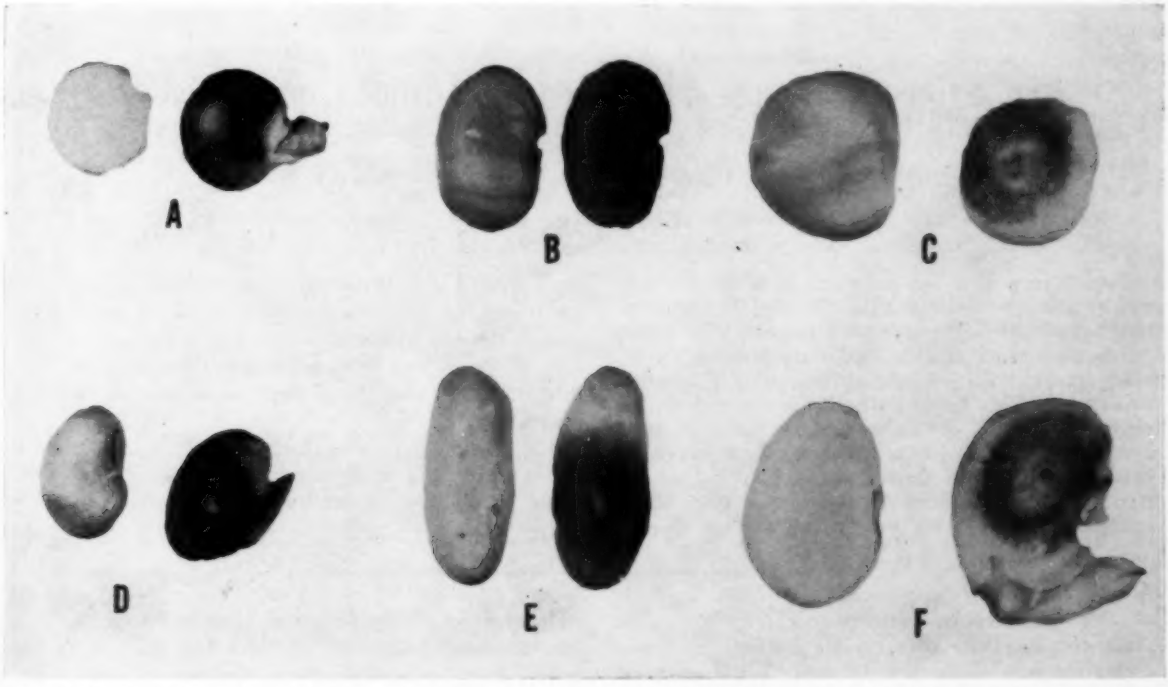

FIG. 1. Seeds of 5 species of legumes showing the discoloration resulting from artificial inoculation with 3 species of *Cercospora*. Paired seeds are of the same legume; the one on the left was not inoculated, the one on the right was inoculated. A) White-seeded yellow lupine. B) Ogden soybean. C) Alabama White lupine. D) Lady Pea cowpea. E) White Creaseback snapbean. F) Thorogreen lima bean.

stizolobii from velvet bean, and *C. zebrina* from *Trifolium ambiguum*.

Two *Cercospora* species isolated from grasses also discolored the seeds of these legumes. These were *C. penniseti* from pearl millet and *C. sorghi* from Johnson grass, Sudan grass, and sorgo. One isolate of *C. sorghi* from Johnson grass failed to produce purple discoloration of the seeds of any of the 5 legumes inoculated with it. This isolate also failed to discolor soybean seeds as previously reported. The other isolate of *C. sorghi* that had failed to discolor soybean seeds discolored the seeds of snapbean, lima bean, white lupine, and cowpea but failed to discolor the seeds of white-seeded yellow lupine. No explanation can be given for this behavior.

The discoloration that developed when legume seeds were plated on agar in Petri dishes and inoculated with cultures of *C. kikuchii*, *C. stizolobii*, and *C. sorghi* is illustrated in Figure 1. Similar symptoms were produced by these 3 species of *Cercospora*, as well as by the others tested.

DISCUSSION AND CONCLUSIONS.—Purple stain of soybean and other legume seeds may be produced artificially by inoculating the seed with Cercospora isolates from a variety of leguminous and nonleguminous hosts. Wounding the pericarp apparently is not necessary, as the fungi penetrate the uninjured seed coat and produce the purple discoloration within 5-6 days after the inoculations are made.

Most of the Cercospora isolates used in this study produced a purple discoloration in potato-dextrose agar, but *C. sojina* from soybean, 1 isolate of *C. sorghi* from Johnson grass, and a *Cercospora* sp. from *Strophostyles* sp. did not. These 3 isolates also failed to produce purple stain on any of the seeds upon which they were tested. These results suggest that a correlation exists between the ability of an isolate to cause discoloration in potato-dextrose agar and its ability to discolor seeds.

Threshed seed may be used for studying the purple stain reaction with *Cercospora* species, provided the seed is surface sterilized and plated on agar at least 48 hours prior to inoculation. In some cases, fungi grew out of the surface-sterilized seeds after the 48-hour preinoculation period. These were identified as belonging to the genera *Alternaria*, *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium*, and *Helminthosporium*. This suggests that with some seed lots, a 72-hour preinoculation period may be needed to eliminate more seeds infected with fungi.

The results presented show that purple stain of soybean seeds and of seeds of other legumes may be obtained in Petri dishes in the laboratory by inoculating such seeds with isolates of Cercospora from a number of leguminous and nonleguminous hosts. It would seem, therefore, that the purple stain of soybean seeds is not a specific reaction to *C. kikuchii*, but is a generalized reaction that may be produced by a number of different species of *Cercospora*. There is no evidence to date, however that these other species are a cause of the purple stain of soybean seeds in the field.

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FLYING APHID POPULATIONS IN SOUTHERN CALIFORNIA CITRUS GROVES AND THEIR RELATION TO THE TRANSMISSION OF THE TRISTEZA VIRUS

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SUMMARY

Populations of flying aphids in southern California citrus groves from 1951 to 1954 were determined by trapping with sticky boards. The green citrus or spirea aphid, *Aphis spiraecola* Patch, made up about 85 per cent of the catch. The melon or cotton aphid, *Aphis gossypii* Glover, constituted only 3 per cent of the catch, but the 35,000 of these aphids recorded as flying to each citrus tree each year in the area of the most rapid spread of the tristeza (quick decline) virus were more than enough to account for the spread of this disease even though the transfer of an average of 5600 melon aphids was required to produce each infection in the experimental work. The spread of tristeza in the groves seldom exceeded the rate of 2 new infections each year from each diseased tree present.

The tristeza virus was shown to be nonpersistent in its vector and to be carried only by the melon aphid in this area, at least at a demonstrable level of efficiency.

In their 1951 report, Dickson et al (2) estimated the relative abundance of various aphid species living on citrus trees in southern California. For a study of transmission of the tristeza (quick decline) virus (*Corium viatoris* Klotz), the most important data are counts of flying aphids. Such data would provide information on the number of aphids that fly from a diseased tree to healthy trees each year. To provide such information, aphid traps were maintained in

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TABLE 1.—*Catches of green citrus aphid and melon aphid on sticky board traps set at various locations on orange trees in southern California (Covina and East Highland), 1952*

Height of traps from ground (ft.)	Green citrus aphid			Melon aphid		
	Total number caught[a]	Percentage of total	Percentage of average	Total number caught[a]	Percentage of total	Percentage of average
	Leeward (east) side of tree					
1	2,247	15.4	92.4	635	43.3	259.8
5	3,831	26.3	157.8	259	17.7	106.2
12	4,128	28.3	169.8	142	9.7	58.2
	Windward (west) side of tree					
1	1,269	8.7	52.2	253	17.2	103.2
5	1,629	11.2	67.2	137	9.3	55.8
12	1,407	10.1	60.6	39	2.7	16.2

[a] Caught on 2 traps in 32 days.

orange trees located throughout the southern California citrus area from 1951 to 1954.

SAMPLING.—Populations of flying aphids were sampled by the use of sticky board traps as developed by Kaloostian and Yeomans (3) for pear psylla scouting. These traps were hung in the periphery of orange trees about 5 ft. from the ground. Two were placed in each tree, 1 on the west and 1 on the east side.

It was soon noticed that the traps on the east (leeward) sides of the trees usually caught more aphids than did the traps on the west (windward) sides. In a further experiment, traps were placed 1, 5, and 12 ft. above ground on 15-ft. orange trees in heavily infested groves at Covina and East Highland. The traps were left for a few days, then were brought to the laboratory, and the aphids removed and identified (Table 1). About 70 per cent of each of these 2 species of aphid recorded were taken on the leeward sides of the trees. The green citrus aphid, *Aphis spiraecola* Patch, showed a rather even vertical distribution, with slightly more caught at the higher elevations than near the ground. The reverse was true of the melon aphid, *Aphis gossypii* Glover; both leeward and windward sides showed much higher concentrations near the ground.

When counts of both species were combined, traps 5 ft. above ground on both windward and leeward sides of the trees gave a relatively accurate sample for the tree (96.7 per cent of the average of 6 locations). The catches of the green citrus aphid at this height were too high (112.5 per cent of average), whereas the catches of the melon aphid were too low (81.0 per cent of average).

It was desired to sample the actual number of aphids flying to an area of the surface of a citrus tree equal to that of a trap (50 sq. in.), so that the total number flying to the tree could be calculated. It seemed probable that the yellow traps were more attractive to the aphids than an equal surface area of natural tree canopy. As 1 method of testing this attractiveness, frames were built to hold 12 of the 5 × 10 in. yellow sticky board traps used in the survey. This made it possible to compare giant traps 20 in. wide and 30 in. high, having a total area of 600 sq. in. and 100 in. of outside edge, with single traps having a total area of 50 sq. in. and 30 in. of outside edge. Two traps in the center of each giant trap had no outside edges (Table 2). The 3 aphid species sampled by this method, especially the green peach aphid, *Myzus persicae* (Sulz.), responded differently to the 2 kinds of traps. The single boards caught 4.3 times as many green citrus aphids as did the traps comprising the giant trap (averaged) and 8.7 times as many as did the center traps; the corresponding figures for melon aphids were 2.9 and 6.5 and for green peach aphids, 1.0 and 1.6. If the center boards of the groups are considered to have made catches equal to the number of aphids flying to an equal area of tree canopy, the yellow boards attracted green citrus aphids from an area 6.75 in. beyond their edges, melon aphids from 5.3 in., and green peach aphids from 0.9 in.

In another experiment, artificial orange leaves made of aluminum and lacquered yellow were used. These were covered with sticky material and wired to orange trees beside natural orange leaves of the same size that also were covered with the adhesive. The leaf pairs were picked off after 2–4 days and the aphids counted on each leaf. (Only the green citrus aphids were counted.) Sixty pairs were set out for 4 periods totaling 12 days. During this time the yellow artificial leaves caught 9754 aphids, while the natural leaves caught 2436 aphids. The yellow artificial leaves thus caught 4.0 times as many aphids as did the natural leaves.

A number of orange trees of average size were measured and found to average 14.25 ft. in height and 50.42 ft. in circumference. On the assumption that an orange tree is shaped like a cylinder topped by a

TABLE 2.—*Average catches of 3 species of aphids on sticky board traps[a] hung alone compared with those on traps hung in groups of 12 and with those on the 2 center traps of such groups*

Species	Average number of aphids per trap		
	Traps hung individually	Traps in groups of 12	
		All 12	Center 2
Green citrus aphid	177.2	41.7	20.2
Melon aphid	18.0	6.1	2.7
Green peach aphid	35.2	36.4	22.2

[a] Traps hung on orange tree canopy surface in southern California, April 21 to May 31, 1951.

hemisphere, it was calculated that these trees had an average outside area of 718.37 sq. ft. or 103,445 sq. in. This is 1033 times the area of the 2 traps (100 sq. in.). By a slight reduction in trap area to account for insects rubbed off in the carrying box, an even figure of 1100 was obtained and used. That is, the traps hung in the foliage covered 1/1100 part of the tree surface. From the data obtained by using artificial leaves, it was assumed that the traps were 4 times as attractive as leaf-covered tree surface. Thus it was calculated that for each aphid caught on the 2 traps, 275 had actually flown to the tree. This figure is as good an approximation as could be obtained, although it is recognized that differences in tree size and in the behavior of various aphid species may make it far from exact in many cases.

SURVEY.—From 1951 to 1954, sticky board aphid traps were maintained on orange trees in 17 locations in southern California. The traps were changed each week, and the exposed traps were brought into the laboratory. All the aphids (or an aliquot) were removed, washed, treated to relax and restore turgidity, and identified. Only the 5 aphid species most commonly found breeding on citrus here were identified. The rest were counted and lumped as "others."

On the basis of distance from the coast and therefore on climate, the area surveyed was divided into the following 4 districts: interior, intermediate, coastal plain, and coastal. Results from these 4 districts are shown in Table 3. Since citrus aphids in California are able to live only on immature leaves or on other immature tissues of citrus, the aphid populations generally follow the growth cycles. The greatest population occurs in late March, April, and May with the spring growth cycle. Since the data are for flying aphids, they show some lag behind the breeding populations and even more lag behind the tree growth. There were sometimes smaller peaks in July or August and in November or December.

Aphis gossypii Glover, the melon or cotton aphid, is ordinarily dark gray when it occurs on citrus. It breeds on many hosts and is very widespread. In southern California, citrus trees, particularly orange, are among its preferred hosts. On the average, it made up 3 per cent of the aphids taken on the traps. The number computed as flying to an orange tree each year ranged from 35,600 in the intermediate district to 3,200 on the coast. Since this aphid is the demonstrated vector of the tristeza virus in southern California, it is not surprising that the intermediate district around Covina and Azusa was the area in which this disease spread most rapidly.

Aphis spiraecola Patch, the green citrus or spirea aphid, is apple-green in color and in southern California is practically confined to citrus, although it also infests spirea, apple, peach, *Viburnum* spp., *Pyracantha* spp., and *Cotoneaster* spp. On the average it made up more than 85 per cent of the aphids caught flying to the orange trees. This is the aphid species that causes almost all the direct aphid injury to citrus in California and Florida. The records showing 600,000–800,000 green citrus aphids flying to each citrus tree per year in certain districts explain the difficulty in controlling this species with insecticides. Green citrus aphid populations were highest in the early summer, but some of these aphids were present at all seasons. Populations were higher in the intermediate and coastal plain districts than in the interior or on the coast. This species has not been shown to carry the virus of tristeza in California.

Myzus persicae (Sulz.), the green peach aphid, is yellow-green to pinkish and breeds on a large number of plant species. It is a very active aphid and has been shown to carry a great many plant viruses, but not the virus of tristeza. This aphid occurred throughout the year and was somewhat more numerous in the interior than near the coast. The green peach aphid breeds freely on several weed plants commonly found in citrus orchards. Probably, therefore, a great many of the specimens caught came from plants other than citrus.

Toxoptera aurantii (Fonsc.) has been called "black citrus aphid" in the United States and "brown citrus aphid" in most other countries where it occurs. A move is now being made to establish the name "brown citrus aphid" in this country. This is appropriate since most specimens are mahogany brown in color. The name "black citrus aphid" is applied in most countries to *Aphis citricidis* (Kirkaldy), the aphid that carries the tristeza virus so efficiently in South America (5) and elsewhere but does not occur in the United States. *T. aurantii* lives on citrus and camellia in southern California and is more common near the ocean than in the interior, as is to be expected from its generally tropical distribution. It has not been possible to show that *T. aurantii* is a vector of tristeza virus in California.

Aphis medicaginis Koch, the cowpea aphid, is the blackest aphid species found on citrus in this area. Old apterous females are shiny black. This aphid breeds on many kinds of plants and tends to be more numerous in the interior districts than near the coast. This same tendency becomes exaggerated in the desert and San Joaquin Valley districts, where the cowpea aphid is the most common aphid on citrus. This species does not seem to be a vector of the tristeza virus.

Other aphids, few of which ever breed on citrus, made up about 6.5 per cent of the total catch. These insects came from nearby weeds and crops, the majority coming from grasses, crucifers, or *Malva* sp. Other species occasionally found breeding on citrus are the potato aphid, *Macrosiphum solanifolii* (Ashm.); the foxglove aphid, *Myzus solani* (Kalt.); the sunflower aphid, *Aphis helianthi* Monell; and the bean aphid, *Aphis fabae* Scop.

Generally, the melon aphid population was scattered more evenly throughout the year than that of the green citrus aphid. In relation to possible reduction of rate of spread of the tristeza virus by intensive control of citrus aphids by insecticides, it is interesting to note

TABLE 3.—*Average number of aphids of various species flying per month (1951-1954) to single orange trees in 4 southern California districts*

	Aphis goss.	*Aphis spir.*	*Myz. pers.*	*Tox. aur.*	*Aphis medic.*	Other spp.	Total
Interior District (Corona, Riverside, Redlands, East Highland, Cucamonga):							
Jan.	109	1,897	934	0	27	1,048	4,011
Feb.	0	826	3,968	0	118	2,677	7,589
Mar.	220	16,582	6,981	0	294	5,064	29,141
Apr.	3,148	113,890	7,370	82	696	8,827	134,013
May	5,697	176,580	10,399	55	748	5,756	199,235
June	146	4,162	311	36	91	788	5,534
July	884	3,190	32	0	0	829	4,935
Aug.	682	2,535	0	0	82	1,200	4,499
Sept.	2,182	1,338	55	0	246	2,109	5,930
Oct.	2,347	2,309	36	18	356	2,970	8,036
Nov.	7,641	2,996	247	0	605	3,025	14,514
Dec.	1,979	2,859	192	0	82	604	5,716
Total	25,035	329,164	30,525	191	3,345	34,893	423,153
Per cent	*5.92*	*77.79*	*7.21*	*0.05*	*0.79*	*8.25*	
Coastal Plain District (LaHabra, Fullerton, Anaheim, Tustin, Atwood)							
Jan.	0	13,914	1,703	27	0	1,566	17,210
Feb.	0	9,579	5,362	37	55	4,739	19,772
Mar.	206	76,622	4,461	344	201	5,746	87,580
Apr.	1,839	279,368	1,210	1,251	220	3,317	287,205
May	903	91,647	1,838	254	72	3,685	98,399
June	531	13,824	18	18	0	239	14,630
July	230	18,415	46	0	55	944	19,690
Aug.	51	22,027	92	0	0	1,188	23,358
Sept.	18	8,506	55	0	27	1,063	9,669
Oct.	18	5,335	36	54	81	807	6,331
Nov.	27	50,983	219	0	54	742	52,025
Dec.	0	41,028	357	0	0	549	41,934
Total	3,823	631,248	15,397	1,985	765	24,585	677,803
Per cent	*0.56*	*93.13*	*2.27*	*0.29*	*0.11*	*3.63*	

	Aphis goss	*Aphis spir.*	*Myz. pers.*	*Tox. aur.*	*Aphis medic.*	Other spp.	Total
Intermediate District (LaVerne, Azusa, Covina, Irwindale)							
Jan.	1,477	46,369	2,886	0	240	4,914	55,886
Feb.	344	11,389	8,856	0	69	6,462	27,120
Mar.	2,486	159,156	11,939	34	1,398	12,553	187,548
Apr.	9,556	336,909	5,619	1,786	917	12,031	336,818
May	2,920	99,744	2,062	309	0	4,960	109,995
June	4,515	6,691	138	0	160	1,718	13,222
July	2,887	58,896	137	0	23	3,415	65,358
Aug.	3,701	38,911	17	0	298	7,086	50,013
Sept.	2,498	9,372	46	92	789	8,617	21,414
Oct.	2,038	7,860	184	0	653	5,729	16,464
Nov.	1,303	12,922	239	68	548	2,372	17,452
Dec.	1,854	20,623	995	68	103	1,305	24,948
Total	35,561	808,842	33,118	2,357	5,198	71,162	956,238
Per cent	*3.72*	*84.59*	*3.46*	*0.25*	*0.54*	*7.44*	
Coastal District (Costa Mesa, San Juan Capistrano, Capistrano Beach)							
Jan.	0	1,511	411	46	46	870	2,884
Feb.	31	826	2,047	0	0	2,902	5,806
Mar.	260	3,659	1,452	275	61	3,026	8,733
Apr.	826	55,254	939	3,368	153	4,376	64,916
May	1,703	51,526	206	252	61	3,179	56,927
June	0	2,475	0	0	0	336	2,811
July	138	10,947	69	46	0	473	11,673
Aug.	115	9,196	31	92	138	726	10,298
Sept.	0	1,374	31	31	0	459	1,985
Oct.	0	8,586	92	31	46	582	9,337
Nov.	92	2,933	641	257	46	1,099	5,086
Dec.	0	4,216	458	92	46	457	5,269
Total	3,165	152,503	6,377	4,508	597	18,575	185,725
Per cent	*1.70*	*82.11*	*3.43*	*2.43*	*0.32*	*10.00*	
Average of 4 districts							
Total	16,896	480,439	21,354	2,260	2,476	37,304	560,730
Per cent	*3.01*	*85.68*	*3.81*	*0.40*	*0.44*	*6.65*	

that the complete suppression of citrus aphids during the 2 months that they are most numerous (usually April and May) would reduce the total annual population of green citrus aphid in all districts by 69.6 per cent, but would reduce the melon aphid population by only 55.1 per cent. This tendency is most marked in the 2 interior districts, where this program would reduce green citrus aphids by 74.8 per cent, but would reduce melon aphids by only 34.5 per cent. Since it is impossible to obtain such a level of efficiency in field treatments, it is doubtful that measurable reduction in field spread of the virus could be obtained by insecticides. This sort of program would be further hindered by the reluctance of the owners of tolerant sweet-on-sweet groves to treat their trees only to protect their neighbors who own susceptible sweet-on-sour groves. Sweet-on-sweet orange trees may be infected with the tristeza virus and serve as sources of inoculum, even though they show no serious symptoms.

THE VECTOR.—The melon or cotton aphid is the only demonstrated vector of the tristeza virus in California. Symptoms reported earlier (2) as having appeared in trees to which treehoppers (Membracids) had been transferred are now known to have been caused by a still undefined disorder and not by tristeza. Test trees for 3 other aphid species, *Aphis spiraecola*, *Aphis medicaginis*, and *Toxoptera aurantii*, have developed tristeza, but a long series of tests with these 3 species failed to show infection significantly above the accidental rate shown by the checks (0.7 per cent). In Florida, Norman and Grant (6) have transmitted tristeza virus from Temple orange by the use of the green citrus aphid. Temple orange is not grown commercially in California, and practically all of our transfers have been from Valencia and Washington Navel orange trees, mostly from Valencia.

TYPE OF TRANSMISSION.—Tests to determine manner of transmission of tristeza virus were conducted with melon aphids as test insects, infected Valencia orange foliage as source of virus, and small grafted sweet-on-sour orange seedlings as test plants. Melon aphids used in these experiments were virus-free since they had been reared on watermelon plants in the greenhouse. In the first of these tests, melon aphids were starved for 2 hours or more, placed on infected plants in lots of 20–25, left there for 5 minutes, and then transferred to healthy test plants. Ninety-five of these tests, in which a total of 2123 melon aphids were used, resulted in 3 infections. The figures show averages of 22.35 aphids per test and of 707.65 aphids transferred for each infection. Results indicate that a short acquisition feeding period is sufficient to allow the melon aphid vector to transmit the tristeza virus.

Another series of tests was made to determine whether or not this virus is retained by the vector for appreciable periods of time. Melon aphids grown on infected plants were transferred to healthy test plants and allowed to feed for 2 hours, then transferred to new test plants and left for 48 hours, and finally transferred to new test plants and left for about a week. Thirty-one tests of this type were conducted, using 23,450 melon aphids. Three infections were obtained; all occurred in plants fed on during the initial 2-hour feeding period. These figures give an average of 756.5 aphids per test and of 7816.7 aphids per infection. Results indicate that the tristeza virus is not retained by the vector, thus classifying it as a nonpersistent virus.

VECTOR EFFICIENCY.—During the course of the study of the transmission of tristeza virus by the melon aphid, a large number of transmission tests were made with colonies of different sizes. All of these aphids had fed for a week or more on diseased orange trees before being transferred to the healthy test plants. The earlier test plants had tops of sweet orange (*Citrus sinensis* (L.) Osbeck) grafted on roots of sour orange (*Citrus aurantium* L.); later ones were lime (*Citrus aurantifolia* (Christm.) Swingle) seedlings. The virus source was Valencia orange in almost all tests. The larger transfers consisted of successive transfers to the same test plants, usually on successive growth cycles, since melon aphids feed freely on orange leaves only while the leaves are immature.

Compared to *Aphis citricidis* the melon aphid is a very inefficient vector of the tristeza virus (Table 4). Bennett and Costa (1) reported that transfers of large colonies (100–300) of *A. citricidis* gave 76.3 per cent infections, whereas in these studies in California only 6.3 per cent infection was obtained with similar colonies of melon aphids. Increasing the number of aphids per transfer increased the percentage of infection, but not proportionally (Table 4). By means of Figure 1, it was calculated that if single aphids were used in transmission tests, 1 transmission in about 407 transfers could be expected. Watson's (7) formula (P =

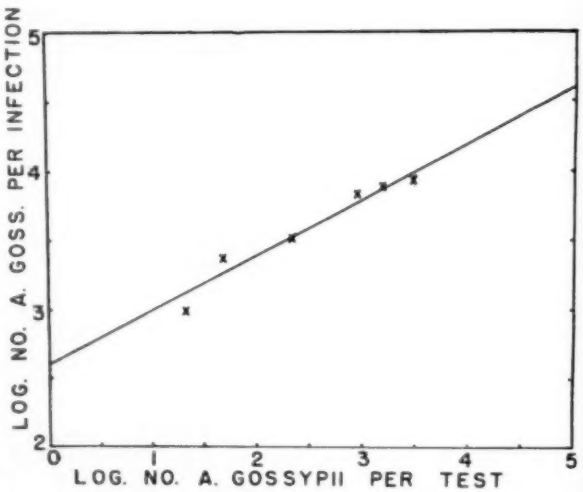

FIG. 1. Log-log graph of results of all tests on the transmission of tristeza virus by the melon aphid, *Aphis gossypii*. Long acquisition and transmission feeding periods were used.

TABLE 4.—*Transmission of the tristeza virus by melon aphids given long acquisition and transmission feeding periods*[a]

No. aphids per test		No. tests	No. aphids	No. infections	Aphids per infection	Percentage of plants infected	Expected percentage of infected plants calculated from indicated probability of infection by single insects	
Range	Average						1 in 407	1 in 979
5-30	21.2	142	3,005	3	1,001.7	2.11	5.04	2.11
31-75	50.0	207	10,335	5	2,071.0	2.42	11.59	4.95
76-500	220.9	412	91,000	26	3,500.0	6.31	41.99	20.10
501-1000	898.9	91	81,800	13	6,292.3	14.29	89.08	59.76
1000-2000	1,619.3	57	92,300	11	8,390.9	19.30	98.15	80.68
2001-4700	3,053.7	41	125,200	14	8,942.9	34.15	99.95	95.50
Total or Average	424.9	950	403,640	72	5,606.4	7.58		

[a] Virus source: Orange. Calculated theoretical values from 2 probabilities of infection by single aphids are included for comparison.

$1-q^x$, where P is the probability of infection by x insects, and q is the probability of noninfection by 1 insect) was applied, using the value of 1 in 407 for single insects or $q = 0.997543$. The calculated values shown in Table 4 do not fit the experimental results. The same formula was therefore used again with the value of 1 in 979 for single insects. This was calculated from the percentage infection (2.11) obtained from the tests with an average of 21.2 insects per test. Again the calculated values for larger insect colonies were much larger than the results obtained experimentally.

Watson's formula is an adaptation of the binominal theorem and is based on the assumption that individuals in a group of potential vectors cause separate and independent infections. In most cases where this formula has been applied to transmission data, the calculations indicate that individuals in large transmission tests are less efficient than similar individuals used singly or in small colonies, a phenomenon observed and discussed by Kirkpatrick and Ross (4). Data in Table 4 show an extreme example of this. Any of the factors that have been offered in explanation of this phenomenon may have been acting: variation in plant susceptibility, feeding interference, changes in the test plant caused by the feeding of large numbers, or possible injection of proteolytic enzymes into the plant by feeding aphids.

NATURAL SPREAD.—The rate of spread of tristeza virus in southern California has varied somewhat from year to year and has been much higher in some localities than in others. The most rapid spread was generally in the intermediate district (Covina, Azusa, etc.), where most orchards were ruined commercially about 5 years after the disease was first observed in them. This amounts to spread at the rate of about 2 new infections each year for each diseased tree present in the orchard, if it is assumed that there would have been about 1 diseased tree in 2 acres before the disease was recognized and that infected trees accumulated. Data for this district (Table 3) show more than 35,600 melon aphids moving to each orange tree each year. Since the number of aphids moving to each orange tree should be roughly equal to the number moving from each orange tree, a vector efficiency in the field of 1 infection for each 17,800 melon aphids moving from 1 tree to another is indicated. This is a much lower efficiency than was obtained experimentally in the present work. This may be explained partially by the breeding and feeding of melon aphids on plants other than citrus. It is suggestive, however, that extrapolation of the line in Figure 1 to the point on the horizontal axis corresponding to tests of colonies of 17,800 melon aphids yields a corresponding indicated efficiency of about 1 infection to 18,000 aphids.

Since Norman and Grant (6) have reported the green citrus aphid to be a vector of the tristeza virus in Florida when Temple orange (a mandarin-orange hybrid) was used as the virus source, there has been a question of whether this species might be acting as a vector of the tristeza virus in California. The green citrus aphid has not been shown to be a vector in California, but computation of the probable vector efficiency required to cause the observed field spread if this aphid had been the vector may be significant. Data from Table 3 show that some 800,000 green citrus aphids fly to (or from) an orange tree in the Covina district each year. The observed field spread could be accounted for if this aphid had a vector efficiency of about 1 in 400,000, or about 4.4 per cent as high as that required of the melon aphid. Since the melon aphid is present and has shown vector efficiency more than sufficient to cause the observed spread, any transmission by the green citrus aphid, if it occurs, must be extremely rare and would be difficult to prove.

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BACTERIAL ZONATE SPOT OF CABBAGE [1]

M. A. Smith and G. B. Ramsey [2]

SUMMARY

A bacterial disease, unlike any previously seen on cabbage on the Chicago market, is characterized by round to irregular, zoned or target-like lesions that occur on outer or second head leaves. The organism proved pathogenic to certain members of the Cruciferae and the Cucurbitaceae as well as to potato, tomato, pepper, bean (green), pea, beet, onion, head lettuce, and witloof chicory. Morphological studies showed that it is a short rod, motile by 2 or more polar flagella, Gram-negative, and not acid-fast. It produces green fluorescent pigment in beef infusion dextrose agar and in Uschinsky's solution.

The morphology, cultural characteristics, biochemical reactions, and pathogenicity of the zonate spot organism were compared with those of *Pseudomonas cichorii* and *Pseudomonas marginalis*. The zonate spot organism and *P. cichorii* were alike pathogenically, and they showed similar morphological, cultural, physiological, and biochemical reactions. They differed from *P. marginalis* in certain biochemical reactions and in pathogenicity. The cause of the zonate spot disease is considered to be *P. cichorii*.

In January, 1953, Florida-grown cabbage with numerous target-like lesions on the outer leaves was observed on the Chicago market. The disease was characterized by round to irregular lesions 2–10 mm in diameter that at first were buff to wood-brown but became seal-brown (7) with age and appeared on any portion of the leaf (Fig. 1, A). Infected areas were firm and pliable but not soft. The second layer of leaves also showed similar lesions, but the remainder of the head was not affected. The vascular system of the stem and leaves was not discolored. Superficially, the lesions resembled those caused by *Alternaria brassicae* (Burk.) Sacc.

Microscopic examination of affected tissues showed that the cells were filled with bacteria (Fig. 1, C). Isolations from these tissues to beef extract dextrose agar plates yielded bacterial colonies within 2 days at 24–26°C. Many of these were pathogenic on cabbage. They were purified by serial dilutions on beef infusion dextrose agar plates at 5 different times before they were used in identification tests. Fungi were not isolated by this method despite repeated attempts.

The disease will hereinafter be referred to as zonate spot. The results of pathogenicity tests, and of studies on morphology, cultural and physiological characteristics, and biochemical reactions of the bacterial isolates are reported. Unless otherwise indicated, the methods used in preparing culture media and in making biochemical and physiological tests are found in the Manual of Methods for Pure Culture Study of Bacteria (3).

PATHOGENICITY.—Nine heads of cabbage obtained on the local market were washed in tap water, rinsed in distilled water, and towelled to remove surface moisture. Inoculations were by 3 methods: 1) 3 heads were inoculated by needle punctures with a 7-day-old beef extract agar culture of the zonate spot organism, 2) 3 heads were cut in half and the cut surfaces smeared with 7-day-old beef extract agar cultures of the organism, and 3) 3 heads were sprayed with a 7-day-old beef extract broth suspension of the organism in sterilized water. Controls consisted of heads

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Investigation conducted by the Biological Sciences Branch, Agricultural Marketing Service, U. S. Department of Agriculture, in cooperation with the Department of Botany, University of Chicago.
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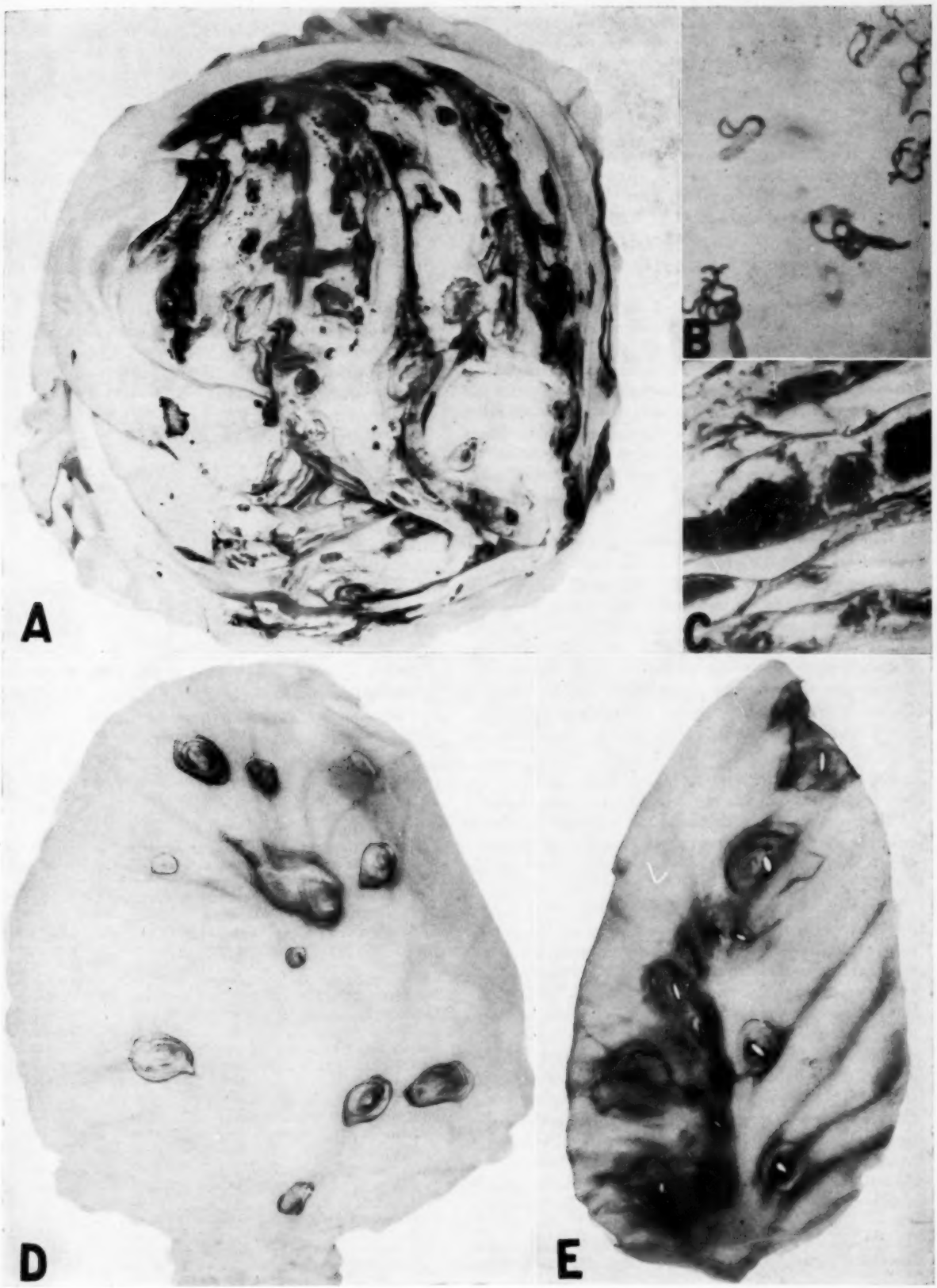
FIG. 1. A) Bacterial zonate spot of cabbage (natural infection). B) Polar flagella of the zonate spot organism. C) Bacteria in parenchyma cells of cabbage. D) Infection following needle-puncture inoculation of cabbage leaf with zonate spot organism. E) Infection following needle puncture inoculation of cabbage leaf with *P. cichorii*.


A
B
C
D
E

injured by sterile needle punctures and cut heads and uninjured heads sprayed with sterilized water. The inoculated cabbage and the controls were placed in moist chambers and kept at room temperature (24–26°C).

Infection of cabbage was generally evident 30 hours after inoculation by needle puncture. Five to 8 days later, the infected areas were circular to oblong or irregularly shaped and varied from 3 to 10 mm in diameter. The lesions were gray to light-brown at first, becoming slightly darker with age and showing the presence of concentric rings (Fig. 1, D).

Bacteria smeared on cut surfaces of cabbage caused decay in 3–5 days. The decayed areas were light-brown to brown in color, but concentric rings, like those from needle-puncture inoculations, were not evident. The organism was readily reisolated in pure culture, by the dilution-plate method, from such tissues as well as from typical zonate spots produced by bacteria inoculated into cabbage by needle-puncture inoculations. A suspension of the organism in water did not cause infection to uninjured heads. Control heads were not infected. These experiments were repeated at various times over a 2-year period with similar results.

Inoculations by needle punctures or by smearing the organism on cut surfaces caused decay of Chinese cabbage, Brussels sprouts, broccoli, cauliflower, kohlrabi, rutabaga, turnip, radish, cucumber, pumpkin, squash, watermelon, green bean, pea, tomato, pepper, potato, beet, onion, head lettuce, and witloof chicory. On these hosts, the decay was slightly soft but not the slimy type produced by *Erwinia carotovora* (Jones) Holland. The zonate type of lesion resulting from needle-puncture inoculation of cabbage was not obtained on any other inoculated host.

Pseudomonas cichorii (Swingle) Stapp and *Pseudomonas marginalis* (Brown) Stevens have been reported to be pathogenic to cabbage (2, 6). When authentic cultures of these bacteria[3] were smeared on surfaces of cut cabbage in this laboratory, decay developed in 4 days at 25°C.

A comparison was made of the symptoms produced by *P. cichorii*, *P. marginalis*, and the cabbage zonate spot organism. Seven-day-old beef extract agar cultures of each of these bacteria were inoculated into heads of cabbage by needle punctures. After inoculation the heads were held in moist chambers at room temperature (24–26°C). In all cases infection was evident after 24 hours. Lesions produced by *P. cichorii* and by the zonate spot organism were similar (Fig. 1, D, E). They were circular to oblong or slightly irregular in shape, were gray to buff in color, and showed ring-like zones similar to lesions on naturally infected cabbage on the market. In contrast, the lesions produced by *P. marginalis* were tan to black, mostly circular, and seldom more than 3 mm in diameter; none showed concentric rings.

Cabbage heads inoculated by needle punctures with 7-day-old beef extract agar cultures of the zonate spot organism or of *P. cichorii* were kept in moist chambers at 6°C and 10°C but did not show infection after 14 days. In pathogenicity tests on various hosts with the 3 organisms, identical results were obtained with the zonate spot organism and *P. cichorii*, whereas *P. marginalis* failed to infect several species susceptible to the others (Table 1).

MORPHOLOGY.—The cabbage zonate spot organism is a short rod with rounded ends, usually occurring singly but occasionally in short chains. Smears from 48-hour-old infections on cabbage contained bacteria averaging 1.9×0.7 μ. The organism is motile: 2 or more polar flagella (Fig. 1, B) were demonstrated by the Leifson method (5). No spores or capsules have been demonstrated. The organism is Gram-negative and not acid fast. It stains readily with crystal violet and carbol rose bengal.

CULTURAL CHARACTERISTICS.—On beef infusion peptone dextrose agar, colonies are round, colorless to cream-white, smooth, convex, and entire. On beef extract peptone dextrose agar slants, growth is smooth, butyrous, and colorless. Growth on beef infusion dextrose agar slants is abundant, filiform to slightly spreading, glistening, and colorless to cream-white. The consistency of some of the isolates is mucoid at first but becomes butyrous. Growth on potato dextrose slants is spreading, white, shining, and butyrous. There is no darkening of the medium. Nutrient broth becomes turbid within 24 hours. There is no well-defined pellicle. Green fluorescent pigment develops in beef infusion peptone dextrose agar, in nutrient broth, and in Uschinsky's and Georgia-Poe media. Growth in Fermi's solution is slight. There is moderate growth in desoxycholate and desoxycholate-citrate agars.

TABLE 1.—*Comparison of the pathogenicity on various hosts of the zonate spot organism with that of Pseudomonas cichorii and P. marginalis*

Host inoculated	Zonate spot organism	*P. cichorii*	*P. marginalis*
Cabbage	+	+	+
Chinese cabbage	+	+	+
Broccoli	+	+	−
Cauliflower	+	+	−
Cucumber	+	+	+
Turnip	+	+	−
Radish	+	+	−
Carrot	−	−	−[a]
Potato	+	+	+
Pepper	+	+	−
Tomato	+	+	−
Onion	+	+	−[a]
Bean (green)	+	+	+
Pea	+	+	+
Beet	+	+	−
Head lettuce	+	+	+
Witloof chicory	+	+	+

[a] Infection of carrot and onion by *P. marginalis* has been reported (4).

[3] Cultures of *P. cichorii* and *P. marginalis* were received from M. P. Starr. A culture of *P. marginalis* was supplied by B. A. Friedman.

The organism is a facultative anaerobe. Gelatin is not liquefied. There is no gas. Neither indole nor hydrogen sulphide is produced. Nitrites are produced from nitrates. Starch is not hydrolyzed. Tests by the Starr (8) method showed that the organism is not lipolytic. Sodium ammonium pectate medium is not liquefied. The organism makes its best growth at pH 6.6–7.2, and its limits for growth are pH 4.8 and 9.2. The maximum temperature for growth is 35°C; the minimum, 5° or less; and the optimum, 26°C. The thermal death point is 52–53°C. In frozen cabbage tissue and in frozen broth cultures (−2°C) the organism remained viable and pathogenic to cabbage for 12 months. Cultures on beef extract agar stabs at 14°C remained viable for 10 months without transfer.

CARBON METABOLISM.—The utilization of 27 carbon sources was tested in a synthetic peptone-free liquid medium (1) to which 1 per cent of the various carbohydrates were added. The various media were adjusted to pH 6.9 with bromcresol purple used as indicator. The criterion for utilization of carbon was the presence of growth as indicated by turbidity and by an increase in acidity. To assure purity, 7 isolates from cabbage were replated 5 times by growing single colonies on beef infusion agar plates. Cultures were incubated at 25°C. The sugars were sterilized by passage through a Seitz filter. The criterion for utilization of organic acids was the presence of growth as indicated by turbidity and by an increase in alkalinity.

Arabinose, dextrose, fructose, galactose, mannose, glycerol, and mannitol were utilized; sucrose, lactose, and maltose were not. Citric, malic, lactic, succinic, tartaric, and acetic acids were utilized; formic acid was not.

The biochemical characteristics of the zonate spot organism, of *P. cichorii*, and of *P. marginalis* were compared (Table 2). These 3 organisms exhibit similar reactions insofar as carbohydrate utilization is concerned, the only exception being the ability of *P. marginalis* to utilize sucrose as a carbon source. The zonate spot organism and *P. cichorii*, however, differ from *P. marginalis* not only in their inability to utilize sucrose but also in their failure to liquefy gelatin, their reaction in litmus milk, their negative reaction in spirit blue agar, and their color when grown in nutrient agar. These differences appear to be sufficient to indicate that the zonate spot organism and *P. cichorii* are distinct from *P. marginalis*.

TABLE 2.—*Comparison of biochemical characteristics of the zonate spot organism with those of P. cichorii and P. marginalis*

Test	Zonate spot organism	*P. cichorii*	*P. marginalis*
Gelatin	Not liquefied	Not liquefied	Liquefied
Nitrates	Reduced	Reduced	Reduced
H_2S	Not produced	Not produced	Not produced
NH_3	Produced	Produced	Produced
Litmus milk	No clearing, alkaline, blue	No clearing, alkaline, blue	Clearing, green
Spirit blue agar	No reaction	No reaction	Blue reaction
Nutrient agar	White	White	Cream to yellow
Uschinsky's solution	Turbid, green	Turbid, green	Turbid, green
Fermi's solution	Slight growth	Slight growth	Slight growth
Sucrose	Not utilized	Not utilized	Utilized

Because of the similar or identical pathogenicity and cultural characteristics of the zonate spot organism and of *P. cichorii*, the writers believe that the bacterial zonate spot herein described is caused by *P. cichorii*.

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THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY IONIZING RADIATION[1]

Ernest Pollard and A. E. Dimond[2]

SUMMARY

Dry purified tobacco mosaic virus when bombarded by deuterons and alpha particles gives a maximum inactivation cross section of 3.5×10^{-11} cm^2. The cross section varies with ion density for very fast deuterons in a manner that requires 3 simultaneous ionizations to produce inactivation. Virus partially inactivated sonically has a lower alpha-particle sensitivity but an unchanged deuteron sensitivity. This indicates that densely ionizing alpha particles can cause transfer of radiation energy over 300 A from one infectious unit to another when rods are aggregated.

X-radiation is more effective than an equivalent amount of energy as 2 Mev electrons. This is probably related to the 3-ionization requirement.

An analysis of the radiation data leads to 2 estimates of virus thickness by independent methods: from inactivations involving deuterons and alpha particles, an effective thickness of 100 A, and from 2 Mev electrons a radius of 57 A and length of 3070 A are obtained. These dimensions differ somewhat from those of tobacco mosaic virus as seen in the electron microscope in which a regular hexagonal cross section of side 87 A and length 3000 A has been described. To account for this difference, it is suggested that the virus has a central radiation-sensitive nucleoprotein core of radius about ⅔ that of the whole virus and that this is surrounded by a much less radiation-sensitive protein capsule.

From data obtained from X-ray, gamma ray, and alpha ray bombardment of several viruses and bacteriophages, Lea (6) concludes that with a few notable exceptions the inactivation of a virus is complete if a primary ionization, or any ionization, occurs within the volume of the virus. If this general conclusion is valid, it offers a method for measuring the size of a virus when the proper conditions of irradiation and ready means of measuring infectivity are available.

The recent experiments by Forro and Pollard (13) on deuteron bombardment of T-1, T-3, and T-7 bacteriophages of *Escherichia coli* and those by Watson (16) on X-ray bombardment of T-2 bacteriophage prove that the radiation sensitivity of these 4 bacterial viruses is such that a single primary ionization has little chance of causing inactivation. Thus, the volume determined from ionizing radiation is definitely less than that of the whole virus as seen in electron micrographs. In addition, the more densely the deuteron ionizes the greater the effect produced, another indication that the virus particle is not inactivated by a single ionizing event.

In tests with tobacco mosaic virus (TMV), Gowen (3) and Lea and Smith (7) have studied the effects of gamma rays, alpha rays, and X-rays on dry virus, and Lea et al (8) have shown that gelatin protects TMV irradiated in dilute solution.

Lea and associates utilized the contrasting patterns of ionization produced by various ionizing agents. For example, the dense swaths of ionization produced by heavy charged particles such as deuterons or alpha particles can be considered as confined to a swath less than 30 A in diameter (13). In contrast, the light ionization produced by a fast electron is effectively random in volume, no trace of the original track being discernible.

In the present study, these patterns were utilized with the objective of determining the nature of the tobacco mosaic virus particle from its behavior upon irradiation. Previous work has been extended also by employing well collimated deuterons of 3.8 Mev energy and 1.75 Mev electrons from an electron accelerator (6). In addition, the effects of varying ionization density and temperature also are reported. All irradiations were made on dry virus. Under these conditions, residual hydration must be considered to be tightly bound, thus placing the action of radiation at the locus of ionization.

EXPERIMENTAL METHOD.—One lot of tobacco mosaic virus (TMV), purified by differential centrifugation, was used throughout these studies. The same amount of concentrated virus preparations was pipetted with a micropipette onto each of 12 replicate ½-in. coverslips. These were dried *in vacuo* and then attached, by means of a little vaseline placed on the reverse side of the coverslip, to copper discs. One disc, bearing a pair of coverslips, served as a control and was not irradiated. The other 10 coverslips were located in each of 10 irradiation positions on a second disc. Each preparation could then be rotated in turn into the path of the deuteron beam from a cyclotron or other source of ionizing radiation. The discs and attached preparations were placed in a bombardment chamber, air was evacuated to 10^{-5} mm Hg pressure, and the cyclotron was turned on. When a suitable current was obtained, an electrical shutter was opened from the control room and the deuterons allowed to strike the

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[2] Biophysics Department, Yale University, and The Connecticut Agricultural Experiment Station, respectively.

single preparation that was in the irradiation position. Various beam currents were employed for various times. The galvanometer that measured the beam was calibrated for each run.

Bombardment by electrons was done by Mr. Marvin Slater, using the Yale Linear Accelerator, and by Dr. D. J. Fluke, using the Brookhaven Van de Graaff accelerator. The general technique of deuteron and electron bombardment has been described previously by Pollard and associates (12, 13).

The 10 virus preparations scheduled for irradiation were handled as 5 pairs of coverslips, each of which received a different radiation dosage. Including the control pair, the 6 dosages were calculated to give predetermined levels of initial virus activity, e.g. 100 (control), 60, 30, 15, 7, and 4 per cent of initial activity.

After irradiation, the coverslips (including the controls) were rinsed with phosphate buffer at pH 7 until the virus was removed. Then each virus solution was brought to a volume 100 times that originally applied to the coverslip. The virus from the pair of coverslips receiving the same dosage of radiation was combined. The virus suspension from the control coverslips was used to prepare a dilution series with a number of infectious particles corresponding to that of the active virus remaining in the irradiated series. Thus in each experiment there were 2 series of virus preparations: a control series at dilutions corresponding, e.g., to 100, 60, 30, 15, 7, and 4 per cent of initial activity, and an irradiated series exposed to 6 dosages of radiation, resulting in comparable percentages of initial activity ranging from 100 to 4 per cent.

The virus preparations were assayed by Holmes' (5) local-lesion technique on half leaves of *Nicotiana glutinosa L.* Opposite leaf halves were inoculated with preparations from the control series and from the irradiated series. The various preparations were otherwise randomized among 6 plants and 6 leaf positions in a pair of Latin squares. Counts of local lesions were averaged over 6 replicates for each preparation. For the control series, the lesion counts were expressed as a percentage of the initial activity and plotted as a function of dilution to give a "calibration curve" for each experiment. This curve was useful because the numbers of lesions that develop are not directly proportional to the numbers of active virus particles in the inocula. The activity of each preparation in the irradiated series, relative to initial activity, was then estimated from the mean lesion count by interpolation on the calibration curve. In this manner, the relative activity of irradiated virus preparations was measured.

In the cyclotron, ionization density was varied by placing metal foils of different thickness (measured as mass in mg/cm^2 of foil area) in the path of deuterons. When this was done, the slower deuterons lost energy more rapidly than fast ones in a calculable manner. The method of stacked foils, a technique used in nuclear physics for the study of relative energy effects, is a very convenient means of measuring this effect.

The individual virus preparations were pipetted onto weighed thin aluminum foils of known equivalent absorption. These were then placed one behind the other to form a stack of 8 or 10 foils separated by brass spacers about 1 mm thick to prevent the preparations from touching. The whole stack was then irradiated by deuterons. Each foil received the same amount of beam except toward the end of the deuteron range, where the effects of straggling and of beam energy inhomogeneity reduced the number of deuterons penetrating to the specimen. Beyond the range of the deuterons there was no bombardment, and specimens placed there were used as controls.

RESULTS AND DISCUSSION.—The survival curve of TMV exposed to deuterons or alpha particles in varying dosage is semilogarithmic, i.e. the curve is linear when the logarithm of percentage survival is plotted against dosage in deuterons/cm^2 (Fig. 1). This has been observed for X-rays and alpha rays by others (3, 6). If the process of inactivation consists of ran-

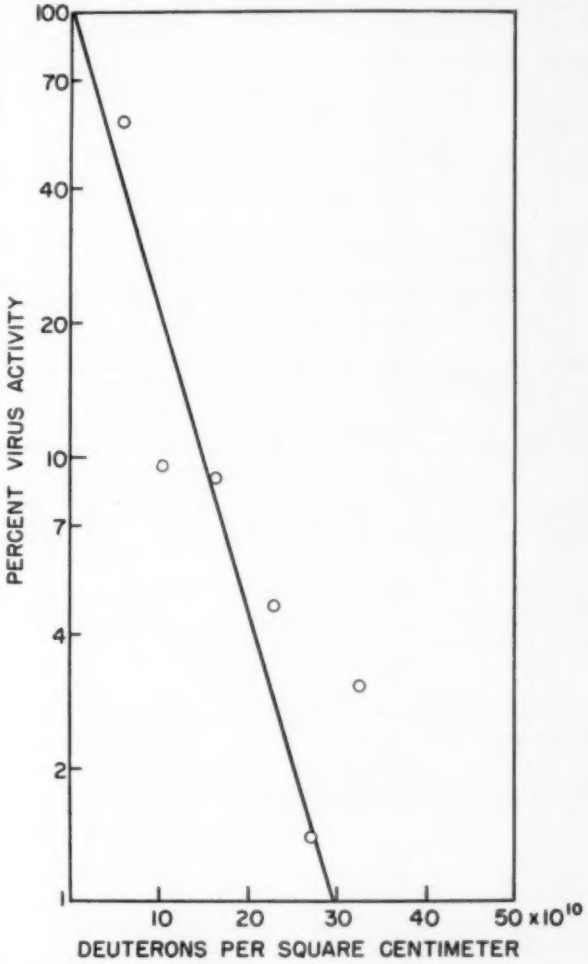
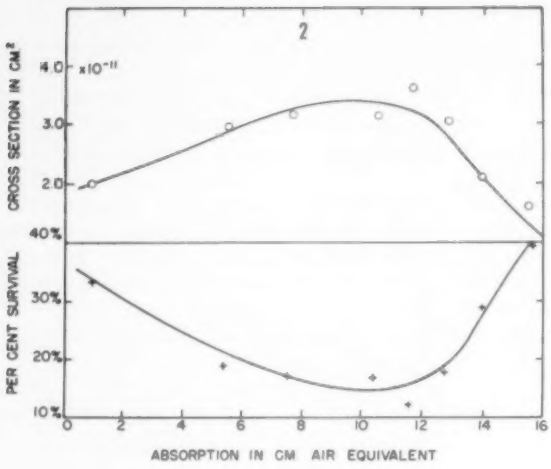


FIG. 1. Plot of percentage virus activity versus dose of deuterons per cm^2. The inactivation is according to the relation $\ln n/n_o = -SD$. This is 1 run.



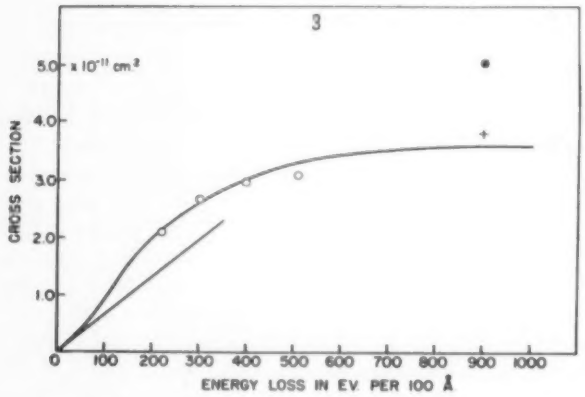


FIG. 2-3.—Fig. 2. Plot of the percentage activity (crosses) versus absorption in the deuteron path for a single stacked-foil experiment. The deduced cross sections are plotted as circles.—Fig. 3. Mean of all stacked-foil runs with deuterons, with allowance for all data taken at lowest ionization density. The effect of varying ionization density is shown. The cross is a point taken with alpha particles on partially sonically inactivated virus. The filled circle is for the normal virus inactivated by alpha particles.

dom passage of a deuteron through a virus particle that presents a target cross section S to each deuteron, then

$$\ln n/n_0 = -SD$$

where D is the number of deuterons/cm² and n/n_0 the fraction surviving (5). The value of the cross section S can be found from this relationship. The cross section can be interpreted as a probability function that in this case has the dimensions of area. When relatively dense swaths of ionization pass through the virus, it is likely that the cross section S can be related to some property of the virus. The value of the apparent cross section was measured to determine whether the deuterons lost a significant amount of energy while passing through the virus. This was determined by measuring the cross section of the virus under varying ionization density.

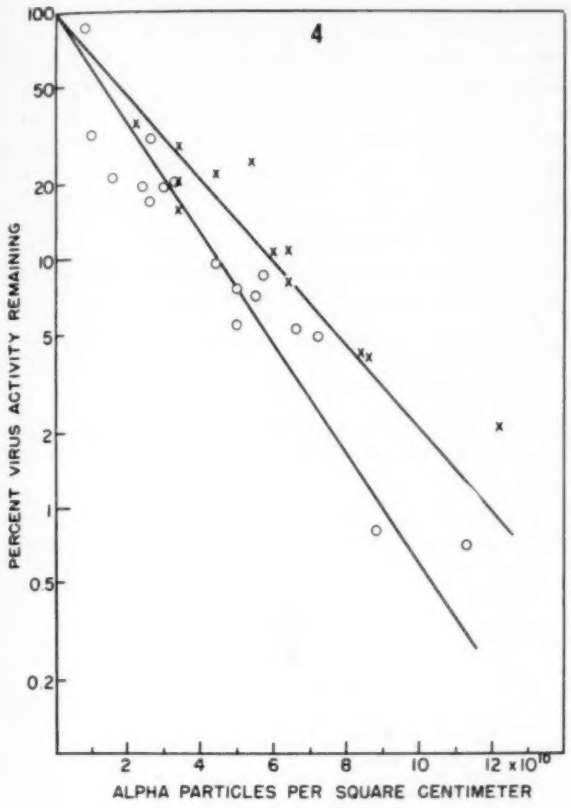
The results of 1 such experiment are shown in Figure 2, in which the percentage virus activity and calculated cross sections are plotted against the absorption in the path of the beam. This technique is advantageous because the effects of varying ion density show very clearly, but it is disadvantageous because any error in the dose is systematic for all points. In principle, a complete logarithmic inactivation curve should be constructed for each determination of cross section. In practice this is too tedious; therefore we determined the fact of logarithmic inactivation at 1 bombarding energy and then assumed that this held for other energies. The average of all cross-section measurements taken for full deuteron energy was used to fix the proper scale for the cross section. The average of 5 runs, in which the stacked-foil method was used, is plotted in Figure 3, where the energy loss per 100 A was calculated from the residual range of the particles as determined from 5 separate curves (as in Fig. 2) and are used as data for the abscissa. The effective cross section is the ordinate.

Because they produce higher ion density than deuterons, alpha particles also were used as bombarding agents. The inactivation of a normal preparation was semilogarithmic (Fig. 4). The cross section so found is plotted as a filled circle on Figure 3. These data do not agree well with the deuteron data. Because this cross section seemed abnormally high and because it is known that TMV preparations can have aggregated particles composed of many multiples of the minimum infective length, it was postulated that some inactivations by very highly ionizing particles could extend from 1 unit to the neighbor and so produce more than 1 inactivation. To check this, the TMV preparation was partially inactivated sonically, a process that greatly shortens the aggregated lengths (10). The sonically inactivated preparation still had considerable infectivity and when bombarded by alpha particles gave the results shown as crosses in Figure 4. This preparation was less sensitive to irradiation than was sonically untreated virus, an interesting result because usually the sum effect of 2 stresses applied to a virus is greater than either alone. The cross section to alpha particles of sonically treated virus is plotted as a cross in Figure 3. This point fits the line through the deuteron points closely.

No significant decrease in cross section occurred when similar sonically treated preparations were bombarded by deuterons. Evidently deuterons do not have the excess energy necessary for migrations from 1 infectious unit to another. A small effect is probable but was not observed.

In contrast to those of deuterons and of alpha particles, the ionization paths of electrons are diffuse and suitable for studying the sensitive volume of a virus. TMV is inactivated by electrons semilogarithmically in proportion to the number of electrons per cm² (Fig. 5). This is expected if the ionization is random in volume. We then find that

$$\ln n/n_0 = -VI$$



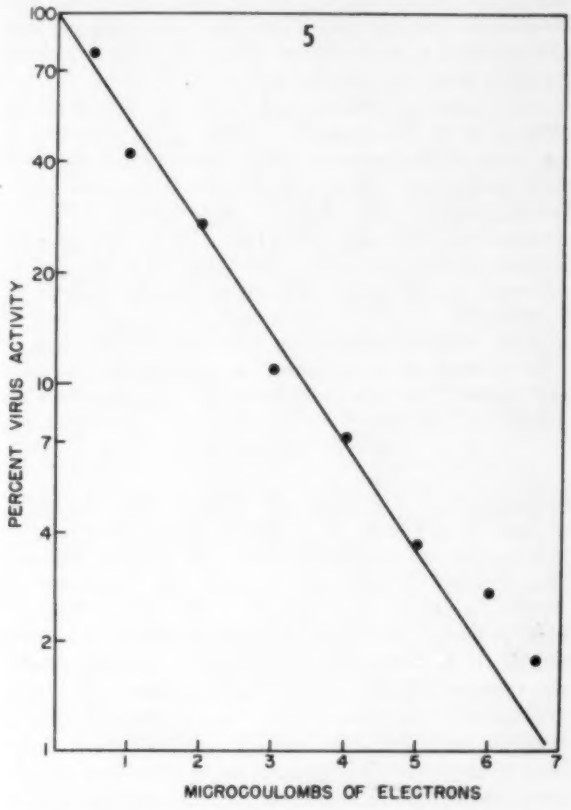


FIG. 4-5.—Fig. 4. Plot of loss of virus activity with alpha particle bombardment. The circles are for normal virus. The crosses for partially sonically inactivated virus. The shorter units in the latter case are less sensitive.—Fig. 5. Loss of activity of virus preparations under fast electron bombardment. One run shown.

where V is the target volume and I is the number of effective primary ionizations per unit volume. In addition, these data can be treated as though electrons were ionizing in swaths of extremely low ion density. Thus the behavior of the cross section versus energy loss curve can be determined near the origin. This was done as follows. The mean of all determinations of the loss of virus activity due to electron bombardment gave a 37 per cent survival figure[3] (mean lethal dose) at 1.25 microcoulombs of electrons, (or $1.25 \times 6.25 \times 10^{12} = 7.81 \times 10^{12}$ electrons). These covered an area of 1.4 cm^2, so that D, the number of electrons per cm^2, was 5.57×10^{12}. Using the relation $\ln n/n_0 = -SD$ where $\ln n/n_0 = -1$ and $D = 5.57 \times 10^{12}$, we deduced $S = 1.8 \times 10^{-13}$ cm^2. The energy loss per 100 A for such an electron is 2.74 electron volts. Thus the slope of the line of Figure 3 near the origin can be established, and is indicated by the straight line drawn from the origin in Figure 3.

The whole curve of Figure 3 now can be interpreted. If a single ionization anywhere in a sensitive target volume causes loss of infectivity, the average number of primary ionizations produced by a particle passing through the target would be pt, where p is the number of primary ionizations per unit length and t is the thickness of the sensitive target. These are subject to fluctuations according to the Poisson law, hence the probability that no ionization will be produced is e^{-pt}. The efficiency of a particle that passes through the sensitive region is therefore not unity, but unity less this probability. The effective cross section S is thus considered to be $S_0 (1 - e^{-pt})$ where S_0 is the actual cross section of the sensitive region.

The curve in Figure 3 does not fit this theory. A probable explanation for this is that more than 1 ionization is necessary for virus inactivation. The efficiency of deuterons when 2, 3, and 4 ionizations are necessary has been derived by Hutchinson (14). The theoretical curve for a requirement of 3 or more simultaneous ionizations is plotted through the experimental points in Figure 3, and it fits the observed points well. Neither the requirement for 2 nor that for 4 ionizations fits as well as that for 3 ionizations, but these alternatives cannot be excluded.

If it is supposed that 3 or more total ionizations (primary or secondary) are necessary at each ionizing event, then the data of Figure 3 yield values of 3.5×10^{-11} cm^2 for S_0 and 100 A for t. In obtaining

[3] For a discussion of the significance of the 37 per cent survival figure, the mean lethal dose, see reference 6, Literature Cited.

the figure 3.5×10^{-11} cm^2, a small correction has been made because of the fact that an alpha particle can produce a large energy release 6 A further from its track than can a deuteron.

Data taken by Wilson and Beekman and cited by Pollard et al (14) indicate that for a fast electron, 23 per cent of the primary ionizations cause 3 or more total ionizations. Then I is 0.23 times the total number of primary ionizations per unit volume. Since 1 electron per cm^2 corresponds to 2.50×10^4 primary ionizations per cm^3 (14), the value of I for the 37 per cent figure[3] previously given is $5.57 \times 10^{12} \times 2.50 \times 10^4 \times 0.23 = 3.2 \times 10^{16}$. Since $n/n_0 = 0.37$ and $VI = 1$, we deduce that $V = 3.13 \times 10^{-17}$ cc.

If we employ these figures to estimate the radius r and length l of the infectious unit (assumed cylindrical) we can put

$$\pi r^2 l = 3.13 \times 10^{-17}$$
$$2rl = 3.5 \times 10^{-11}$$

yielding $r = 57$ A and $l = 3070$ A. The thickness, derived from Figure 3, is 100 A, which is in reasonable agreement with the radius of 57 A just deduced. The deduced length agrees well with the value of 3000 A generally accepted for TMV (17). The volume expected for a regular hexagonal cross section of side 87 A is 5.9×10^{-17} cc, which is nearly double the volume deduced from the present data. This may indicate that the whole virus is not made of equally sensitive material. The nucleic acid, which is 6 per cent of the virus, if contained as a cylinder along the axis, would have a radius of 18 A, a figure not commensurate with our findings. A sensitive core of nucleoprotein of radius about $\frac{2}{3}$ of the average virus radius would fit the data very well. The outer protein would then have much lower sensitivity. This might perhaps be tested with low voltage electrons of limited penetration.

Two other related experiments can be described. The first concerns the combined effect of temperature and deuteron bombardment. Over a range from 20–73°C, no systematic increase in cross section was observed. Adams and Pollard (1) showed that large changes in cross section did not develop below temperatures that will cause thermal inactivation. Since dry TMV is stable up to 90° for several minutes exposure, it is unlikely that 73° is high enough to induce any appreciable thermal effect.

In the second experiment, 2 dry TMV preparations, 1 of purified virus and the other of raw juice, were subjected to rather low voltage X-rays (55 Kv). The 37 per cent figure was around 60,000 r for the purified preparations and somewhat higher for the raw juice. These survival data indicate that low voltage X-rays are considerably more efficient in removing activity than fast electrons, a result previously found by Gowen (4). This is consistent with the requirement for 3 simultaneous ionizations mentioned in the analysis of the data of Figure 3. The presence of many slow electrons in 55-Kv X-rays suggests that a higher proportion of the ionizations are multiple and these X-rays are more efficient than high voltage X-rays.

Of some interest is the transfer of radiation energy observed in the case of alpha particles. Assuming that the radius of the sensitive region is 57 A, the extent to which radiation energy transfer can be made on either side of an infectious unit seems to be 300 A, a very high figure. It must be remembered, however, that an alpha particle can leave as many as 10 primary ionizations behind it in the sensitive region, and it is not improbable that significant energy transfer would result from these multiple primary ionizations.

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THE EFFECT OF SOME ECTOPARASITIC AND ENDOPARASITIC NEMATODES ON THE EXPRESSION OF BACTERIAL WILT IN CARNATIONS [1]

Robert N. Stewart and A. F. Schindler [2]

SUMMARY

Carnation cuttings were inoculated with *Pseudomonas caryophylli* after they had been inoculated with the root-knot nematodes *Meloidogyne hapla*, *M. javanica*, *M. incognita acrita*, *M. arenaria*, and *M. incognita*, and the ectoparasitic nematodes *Helicotylenchus nannus*, *Xiphinema diversicaudatum*, and *Ditylenchus* sp. Treatments with and without root wounds were included as well as parallel treatments without bacteria. Mechanical root wounding increased the rate of wilting in the presence of bacteria but showed no effect in their absence. All 5 root-knot forms and *H. nannus* increased the rate of wilting in the presence of bacteria; only *M. incognita acrita* had a similar effect when no bacteria were present. *X. diversicaudatum* showed no effect on the rate of wilting with or without bacteria. When inoculum containing a *Ditylenchus* species was added, however, there was a decrease in the rate of wilting in the presence of bacteria. Carnation varieties differed in their response to different root-knot species. Resistance to root-knot nematodes was found to be present in some commercial carnation varieties. Both endoparasitic and ectoparasitic nematodes are factors in the bacteria-nematode disease complex of carnations. Most carnation soils examined have yielded mixed populations of several genera of plant-parasitic nematodes. The significant role of the nematodes in the complex is apparently only to provide wounds for entry of the bacterial pathogen.

Until recently there has been little experimental evidence to support the frequent suggestion that plant-parasitic nematodes increase the severity of various fungal and bacterial diseases. In 1901, Hunger (8) described *Heterodera radicicola* (Greeff) Muller, probably a species of root-knot nematode (*Meloidogyne* spp.), as one of several agents capable of causing wounds necessary for entry of the tomato-wilt pathogen. Kalinenko (10) found the same phytopathogenic bacteria within the body cavity of parasitic nematodes as were in the decaying tissue around the nematode lesions in the roots of *Taraxacum kok-saghys* Rodin. Holdeman and Graham (7), using pure cultures of sting nematodes (*Belonolaimus gracilis* Steiner) and of Fusarium (*Fusarium oxysporum* Schlect. f. *vasinfectum* (Atk.) Snyd. & Hans.), showed that the nematode facilitated the development of Fusarium wilt both in susceptible and in resistant cotton plants. They suggested that the nematode did more than merely provide a point of entry for the pathogen. Sasser et al (17) demonstrated that *Meloidogyne incognita acrita* Chitwood increased symptom expression of the black-shank disease of tobacco caused by *Phytophthora parasitica* var. *nicotianae* (B. de Haan) Tucker, and stated that the mechanical wounding of roots in itself did not break down resistance of black-shank-resistant varieties. Lucas et al (13) found that both root-knot nematodes and mechanical wounding increased the development of symptoms of Granville wilt of tobacco, caused by *Pseudomonas solanacearum* E. F. Sm. In support of the nematode-fungus or nematode-bacteria complex concept, a large amount of indirect evidence based on field observations and soil fumigation studies has been presented (2, 3, 5, 6, 9, 16, 19, 20, 21, 22, 24, 26, 28).

Crosse and Pitcher (4) and Vasudeva and Hingorani (25), studying the relationship between bacterial diseases and nematodes, obtained experimental results that suggested a complex but did not eliminate the possibility that the effect might have been produced by the nematodes alone. Several workers (1, 14, 15, 27) either have reported no significant effect of nematodes on the incidence of disease in field tests and laboratory experiments or have presented a confused picture that cast doubt on the complex theory.

In the course of a breeding program designed to obtain varieties of carnations resistant to the bacterial wilt disease caused by *Pseudomonas caryophylli* Burk., it was found that the pathogen was unable to enter intact root systems of susceptible varieties and that wounds made in transplanting healed within 1 week (12). Several methods of root wounding did permit entry, however, and the possibility that plant parasitic nematodes might be a factor in bacterial-wilt incidence and susceptibility was considered even though carnations are grown in greenhouses in steam-sterilized soil. A survey was undertaken, and 9 of 11 commercial growers visited had carnation beds in which the soil was infested with plant-parasitic nematodes, usually of several different species and often in large numbers. Apparently nematodes may present a problem even where greenhouse soils are sterilized. A series of experiments was therefore undertaken to investigate the effect of plant-parasitic nematodes on the development of bacterial wilt in carnations.

MATERIALS AND METHODS.—With the exception of *Ditylenchus* Filipjev, nematodes for inoculation were obtained from cultures of single plant-parasitic species maintained on host plants growing in sterilized soil.

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No attempt was made to exclude the saprophytic nematodes that often contaminate populations established in pots in the greenhouse. For inoculum, soil and roots from these pots were thoroughly mixed. When rooted carnation cuttings were inoculated, a small amount of steamed soil was placed in the bottom of a 2-in. pot and approximately 25 cc of inoculum was added. The rooted cutting was placed on top of the nematode-infested soil and steamed soil was used to fill the pot.

Bacterial inoculum was a 24-hour culture of *P. caryophylli* in Difco nutrient broth. Ten ml of the bacterial suspension was poured onto the soil around each potted carnation in ½ of the treatments. Clonal varieties of carnations were rooted in sterilized medium and then potted in 2-in. clay pots. Nematode inoculations were made at the time of potting and bacterial inoculations 1 week later. Following the bacterial inoculations, the soil temperature was maintained with electric heating cables at approximately 32°C, which is close to the optimum for the rapid development of bacterial-wilt symptoms (12). All pots were plunged in sterilized sand to within approximately ¼ in. of their tops, and the rows were separated by about ½ in.

In all tests, unless otherwise noted, the following 6 standard treatments were applied: 1) control, 2) bacteria, 3) root wounded, 4) root wounded plus bacteria, 5) nematodes, and 6) nematodes plus bacteria. The unit of each treatment in each replication consisted of a row of 10 plants. Tests were designed as randomized blocks with varying numbers of replications. At the time of the bacterial inoculation, the roots of the plants in treatments 3 and 4 were wounded by stabbing through the soil, at a point near the stem, with a wooden pot label.

Three weeks after inoculation with bacteria, the first of 3 weekly readings was taken. Wilted or dead plants were given a score of 3. No attempt was made to determine the cause of wilting or death of every plant. Plants wilted at the second and third readings were given ratings of 2 and 1, respectively, and the experiments were terminated after the third reading. Thus the maximum value possible was always 30. All rating data were subjected to statistical analysis; all references to significant difference mean statistically significant difference.

EXPERIMENTAL RESULTS.—*Endoparasitic forms.*—1) *Individual Meloidogyne spp.*—A total of 1540 carnation plants in 11 replications were used in a test with 4 species and 1 sub-species of root-knot nematodes: *Meloidogyne hapla* Chitwood, *M. javanica* (Treub) Chitwood, *M. incognita acrita* Chitwood, *M. arenaria* (Neal) Chitwood, and *M. incognita* (Kofoid & White) Chitwood. The increase in the rate of wilting in treatments with both bacteria and nematodes over the treatment with bacteria alone (Table 1, Fig. 1) was highly significant for all 5 of these nematodes. Mechanical wounding in the presence of bacteria had the same effect as nematodes. With the exception of those plants inoculated with *M. incognita acrita* there was no significant effect when plants were wounded or nematodes added if no bacteria were present. Statistical analysis showed a highly significant interaction between bacteria and nematode species because of the fact that the nematode species increased the ratings much more in the presence of bacteria than in the absence of bacteria.

TABLE 1.—*Mean weighted values for rate of wilting in carnations inoculated with P. caryophylli and parasitic nematodes*[a]

Treatment	No bacteria	Bacteria
Control	1.3	5.0
Root wound	1.3	17.8
Meloidogyne hapla	1.0	10.5
M. javanica	1.9	14.2
M. incognita acrita	4.9	14.1
M. arenaria	3.5	15.5
M. incognita	3.2	20.6
LSD, 5 per cent = 3.6; 1 per cent = 4.8		
Control	0.4	10.7
Root wound	2.4	25.7
Meloidogyne spp.	3.4	21.9
Nematode inoculum leachate		9.9
LSD, 5 per cent = 3.6; 1 per cent = 4.8		
Control	2	7.8
Helicotylenchus nannus	2	21.8
LSD, 5 per cent = 4.1; 1 per cent = 5.8		
Control	3.7	6.2
Root wound	11.5	25.5
Xiphinema diversicaudatum	2.2	7.3
LSD, 5 per cent = 5.2; 1 per cent = 7.0		
Control	3.5	15.5
Ditylenchus sp.	0.0	6.0
LSD, 5 per cent = 3.8; 1 per cent = 5.5		

[a] Weighted values are the means of treatment totals where a wilted plant was scored as 3 at the first observation, as 2 at the second, and as 3 at the third. Maximum possible rating is 30.

In the preceding tests, 4 carnation varieties were used in 2 replications each, and a fifth (Tetra Sim) was used in 3 replications. The varieties were not randomized since the experiment was not designed to test varietal differences; however, the data (Table 2) show many highly significant differences in varietal response to nematode species.

2) *Leachate from infested soil.*—In this test, the inocula of the 4 species and 1 sub-species of root-knot nematodes were combined. To test for an effect of the inoculum other than nematode action, a treatment that consisted of the leachate from the nematode inoculum was included. This was obtained by thoroughly mixing a portion of infested soil in a bucket of water and allowing it to stand for about 6 hours. The top third of the supernatant water was decanted and used as leachate inoculum. Treatments were replicated 7 times and involved 3 varieties of carnations.

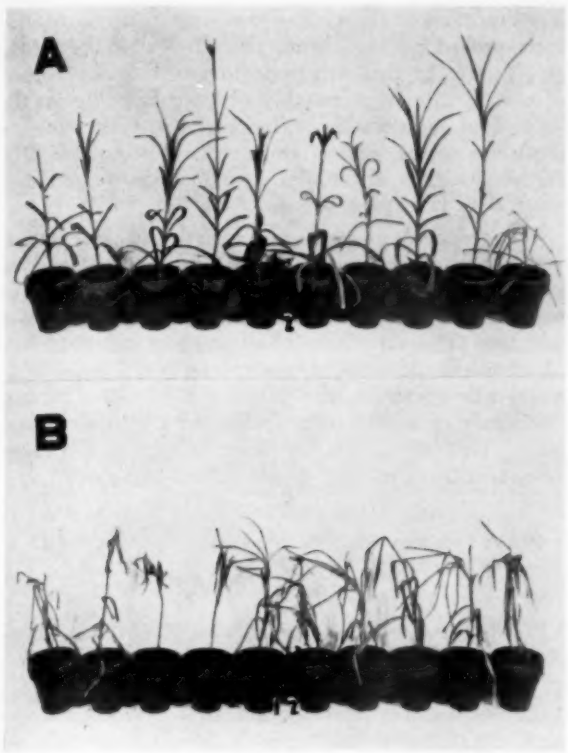

FIG. 1. Carnation variety Tetra Sim inoculated with *Pseudomonas caryophylli* 1 week after potting and photographed 40 days after inoculation. A) Without nematode inoculum. B) Inoculated with *Meloidogyne arenaria* at time of potting.

Results (Table 1) were similar to those previously obtained with individual nematode species. No increase in wilting resulted from addition of nematode-free leachate.

Ectoparasitic forms.—1) *Helicotylenchus nannus* Steiner.—The root-wound treatment was not used in tests with the spiral nematode, but virulence of the bacteria was confirmed in separate inoculations. In 4 replications with the carnation variety Sidney Littlefield, spiral nematodes caused a highly significant increase in the rate of wilting when bacteria were present (Table 1) but no effect in the absence of bacteria.

2) *Xiphinema diversicaudatum* (Micoletzky) Thorne.—When dagger nematodes were added to nonwounded plants of 3 varieties of carnations in 6 replications, the rate of wilting was the same whether or not they had been inoculated with bacteria (Table 1). There was an increase in the rate of wilting when roots were wounded, but the increase was greater in the presence of bacteria, and the interaction was significant at the 1 per cent level.

3) *Ditylenchus sp.*—The nematode inoculum consisted of a *Ditylenchus* sp. cultured in sealed bottles containing, as a food source, the mycelium of the commercial mushroom, *Agaricus campestris* Fr. growing on a substrate of rotted manure. The mixture of manure, fungus, and nematodes was used as inoculum for 4 replications of treatments with the carnation variety Beltsville No. 47-25-31. Virulence of the bacterial inoculum was confirmed in separate tests. In contrast to results with other nematode inoculum, the rate of wilting and death was significantly higher (at the 1 per cent level) in those plants with the bacteria alone in comparison with the plants which had both the Ditylenchus inoculum and bacteria (Table 1). The apparent decrease in the rate of wilting when nematodes were added to the treatments with no bacteria was not significant.

DISCUSSION.—The use of pure populations of single forms both of nematodes and of bacteria or fungi in experiments testing the existence of complexes affecting given plant diseases should not lead to the conclusion that such conditions exist in the field. Steiner (23) has pointed out the need to change from the concept "of a presumed prevalence of single infections and pure populations, to that of actual prevalence of multiple infections and mixed populations." Carnation greenhouse soils have yielded mixed populations of parasitic nematodes; both endoparasitic and ectoparasitic nematodes have been shown to affect the incidence of bacterial wilt; observations by the senior author indicate the existence of several pathogenic races of the bacterial wilt organism; therefore it seems most likely that the usual occurrence is multiple infection by mixed populations of nematodes and bacteria.

There was no evidence in the present study of anything other than a wounding effect on the part of the nematodes, which agrees with the findings of Lucas et al (13). Kelman (11) describes the Granville wilt bacterium as a wound parasite of tobacco, susceptible varieties being infected through natural wounds in the roots and resistant varieties through either mechanically inflicted or nematode-inflicted wounds. In the present tests, the usually significant difference between the unwounded treatments with and without bacteria presumably was due to infection through

TABLE 2.—*Mean weighted values for rate of wilting in carnation varieties inoculated with root-knot nematodes (Meloidogyne spp.) and with Pseudomonas caryophylli*[a]

	Carnation varieties				
	Tetra Sim	Mrs. Guba	Saugus Pink	McCully 310-W	Fuchsia
Control	2.3	1.0	5.0	1.0	6.8
Root wound	12.7	5.8	8.8	5.5	13.5
M. hapla	8.2	4.0	3.5	2.8	9.3
M. javanica	9.3	5.8	7.3	5.8	16.5
M. acrita	6.7	4.8	14.0	5.8	11.3
M. arenaria	11.2	4.3	15.0	3.8	7.5
M. incognita	12.3	10.3	13.8	6.8	16.3

LSD, 5 per cent = 2.3; 1 per cent = 3.0

[a] Values are the means for treatments with and without bacterial inoculum. Weighted values are the means of treatment totals where a wilted plant was scored as 3 at the first observation, as 2 at the second, and as 3 at the third. Maximum possible rating is 30.

a small number of natural breaks in the root system. All carnation varieties tested were susceptible to bacterial wilt when the pathogen was able to invade through some type of root wound. These results with bacterial pathogens are in contrast to those of Sasser et al (17) with the tobacco black-shank fungus, where the effect of mechanical wounding was not the same as nematode injury.

There was generally no direct effect of root wounding on the carnations in these experiments. In the test with root-knot nematodes, however, an increase in the rate of wilting in the absence of bacteria was shown with *M. incognita acrita*. This effect could have been due to a greater concentration of nematodes in the inoculum of this sub-species or to a greater pathogenicity with respect to the host varieties used. The tests reported provide conclusive evidence that root wounding does accelerate the expression of wilt symptoms in carnation plants exposed to *P. caryophylli*.

Two nematode genera failed to increase the incidence of bacterial wilt of carnations. The cultures of *X. diversicaudatum* used were pure cultures of the form proved pathogenic on roses by Schindler (18), and failure of this nematode to affect the incidence of wilt in this case probably resulted from its inability to feed on carnations. The results with Ditylenchus inoculum are of interest and are now being tested in further experiments designed to exclude possibilities other than nematode action.

The response of carnation varieties to inoculation with the bacterial wilt pathogen and different species of root-knot nematodes indicates that resistance to nematodes is present in some commercial carnation varieties. The experiments also suggest that the effect of nematode infestation upon infection by the bacterial wilt organism may prove a good index of susceptibility of a carnation variety to a specific nematode. This would be particularly useful for those ectoparasitic forms that do not induce root galling.

PLANT INDUSTRY STATION
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THE EFFECT OF BOTRYTIS INFECTION ON THE TISSUE OF TOKAY GRAPES [1]

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SUMMARY

Conidia of *Botrytis cinerea*, in nutrient solution on the surface of uninjured Tokay grapes, germinated and produced a macroscopically visible lesion in 36 hours at 20°C. After 60 hours, the lesion was as much as 1 cm in diameter but only 1–2 mm in depth. The skin slipped easily from the pulp when pressure was applied. These symptoms were identical with those shown by infected berries in the vineyard.

The conidia produce germ tubes up to 150 μ in length before an appressorium is formed. The appressorium produces an infection peg less than 1 μ in diameter that penetrates the cuticle. The subcuticular mycelium is usually intercellular and largely restricted to the outermost 5–8 cell layers.

In tissues affected by the fungus, the periclinal walls in the 3–5 outermost cell layers separate more readily than do the anticlinal walls. The epidermal layer usually remains attached to the cuticle, and both are continuous over the lesion.

A water-soluble extract derived from the liquid medium in which Botrytis spores had germinated caused maceration of the skin tissue of thin grape slices. Heated extract produced no effect.

The pectic substances of the intercellular material were more dense between the anticlinal than between the periclinal walls. Disappearance of pectic substances was associated with lesion development.

Botrytis rot, caused by *Botrytis cinerea* Pers., is the most important form of decay of Tokay grapes in the Lodi area of California, destroying a high percentage of the crop in some years when early fall rains wet the mature unharvested fruit. The severity of the disease cannot be predicted, even at the time of the rain, and control is ineffective. In this investigation a study of the histological aspects of the disease has been made so that the organism's mode of infection and its effect on the tissues of the host may be better understood. Such information should be of value in designing control methods.

SYMPTOMS AND SIGNS.—During October, 1948, the mature unharvested fruit was wetted by rain and remained wet for about 30 hours. The temperature ranged from 6° to 21°C during the week following the rain. On the fourth day after the rain, the skin of infected berries cracked easily when pressed lightly, and small macerated areas up to 1 cm in diameter slipped from the firm pulp beneath (Fig. 1, B). No evidence of the lesions could be detected until after the skin had cracked, probably because the red pigment of the subepidermal cell layers masked any discoloration. By the following day, the weakened skin of some of the berries had cracked spontaneously (Fig. 1, C). The lesions often involved ½ of the surface of the berries, yet the tissue was macerated to a depth of less than 1 mm. These observations agree with those of Barretto (2); however, he further observed that the cracks that occurred in the epidermis were largerly confined to the area of contact between the berries. In the present study no such consistent pattern of occurrence was observed. Du Plessis (6) described the symptoms of slipskin as a crisp cracking of the skin, which slipped off easily at the slightest pressure and exposed a normal pulp. He considered this condition to be an early manifestation of Botrytis infection. Sharply marked brownish areas were easily detected on white-skinned varieties. These areas were neither soft nor sunken, a symptom quite different from that produced by other pathogens.

On the seventh day, signs of the pathogen were visible at the site of the cracks as compact gray ridges and tufts of conidia and conidiophores 1–4 mm in height (Fig. 1, D). Pacottet (11) found that the primary fructifications appeared at the edges of the cracks in the pellicle, a term he applied to the epidermis and a few layers of subepidermal cells. In this paper the term (skin) will include the epidermis and 2–4 layers of subepidermal cells.

By the ninth day the gray-mold or bunch-rot stage had developed: groups of infected berries, usually in the interior of the cluster, were matted together by the fungus mycelium (Fig. 1, A, E).

MODE OF INFECTION.—During field studies, lesions often were found on exposed areas of berries where contact infection was improbable, an indication that Botrytis may have gained entrance through microscopic injuries or by direct penetration. Müller

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This account is composed of a portion of a thesis submitted to the Graduate Division, University of California at Berkeley, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, and in addition makes use of part of a study made under the Research and Marketing Act of 1946 while the writer was an Agent (Plant Pathologist) of the U. S. Department of Agriculture at the Horticultural Field Station, Fresno, California. Grateful acknowledgment is made to W. T. Pentzer, W. R. Barger, and W. S. Ballard (retired), of the U. S. Department of Agriculture, to Wm. B. Hewitt, of the University of California, for helpful suggestions during the course of this investigation, and to Mrs. Margery Mann and Lorne Hardaker who prepared the figures.

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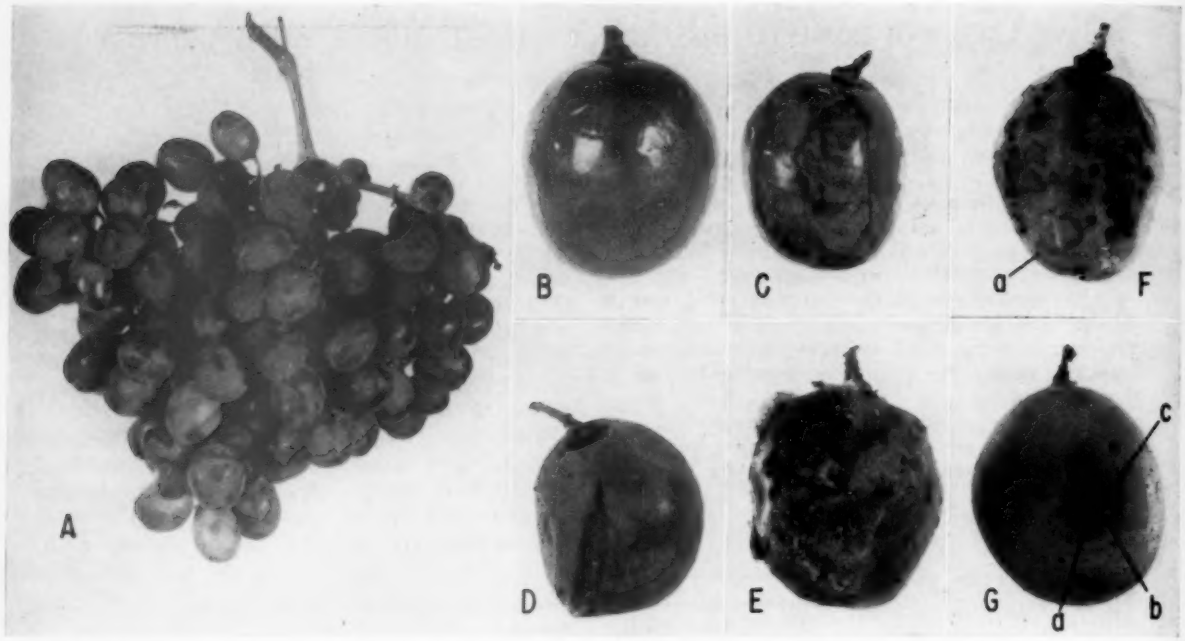

FIG. 1. Tokay grapes infected by *B. cinerea*. A-E) Infected naturally in the vineyard. A) Bunch-rot stage in a cluster of grapes. ×½. B, C) Slipskin, or early stage. D, E) Gray-mold, or bunch-rot, stage. F, G) Grapes inoculated with conidia and kept moist for 48 hours at 20°C. F) Single and composite lesions, 1–4 mm in diameter. The skin over one such composite lesion has split (a). G) One lesion shows 3 zones of discoloration of the subcuticular tissue: inner (a), intermediate (b), and outer (c). ×1½.

Thurgau (10) concluded that infection occurred directly through uninjured skin; however, Guillon reported that the germ tube usually entered through insect punctures or other wounds (8). Brown and Harvey demonstrated that the germ tube of *B. cinerea* mechanically penetrated the epidermis of onion scales and Eucharis leaves (5).

Mature Tokay grapes that had been in cold storage for 10 weeks at 32°F were surface sterilized for 3 minutes with a calcium hypochlorite solution containing 3000 ppm of chlorine, then rinsed with sterile water. They were inoculated with a conidial suspension of *B. cinerea* in turnip juice, prepared as described by Brown (3), and incubated at 12°C while wet. After 18 hours, several berries were partly dried, and then sprayed with Haupt's gelatin fixative and finally with Rawlins' (13) No. 2 formalin-acetic-alcohol killing-fixing solution (FAA). The sprayed berries were allowed to dry for 10 minutes, then thin slices of the grape surface were taken and immersed in Rawlins' No. 2 FAA solution. Additional lots were withdrawn and likewise treated at 6-hour intervals through 48 hours.

The fixed tissue was dehydrated, infiltrated, and imbedded with paraffin according to the tertiary butyl alcohol method of Johansen (9). Sections perpendicular to the surface and 10 μ thick were stained according to the iron-alum haematoxylin schedule of Rawlins (13), except that after the sections had been destained with ferric ammonium sulfate, they were run up through the ethyl alcohol series and stained for 10 minutes in a saturated solution of Sudan IV in 95 per cent ethyl alcohol. After excess stain was blotted from the slides, they were plunged directly into distilled water, and glycerin jelly was used to make permanent mounts.

Microscopic examination showed that the distal portion of the conidial germ tube develops into an appressorium that is closely appressed to the cuticle (Fig. 2, A). An infection peg, which is less than 1 μ in diameter, is produced from the underside of the appressorium and penetrates directly through the cuticle, after which it enlarges into a subcuticular and intercellular mycelium (Fig. 2, B, C, D, E, F). In sections taken early in the infection process (Fig. 2, B, C), appressoria stained much more than in those taken later (Fig. 2, E, F), an indication that the appressoria become depleted of protoplasm as the infection process progresses.

Small areas of skin were peeled from the killed and fixed material, washed for 24 hours, then immersed in an enzyme extract prepared by the method of Brown (3). After 12 hours, the extract had macerated the tissue to the extent that all subepidermal tissue could be separated from the epidermis by manipulation with a camel's-hair brush. The epidermis was mounted, with the cuticle uppermost, on slides and stained with cotton blue.

Observations on this material (Fig. 2, G, H, I), which could be viewed from above, confirmed the previous observations. The distal portion of the germ tube enlarges appreciably, both in width and height, to

form the appressorium. More than 1 germ tube may be produced by a conidium (Fig. 2, G). Although this condition, even to the extent of a well-defined appressorium on each of at least 2 germ tubes from 1 conidium, was observed frequently, multiple infections from 1 conidium were not observed; however, there

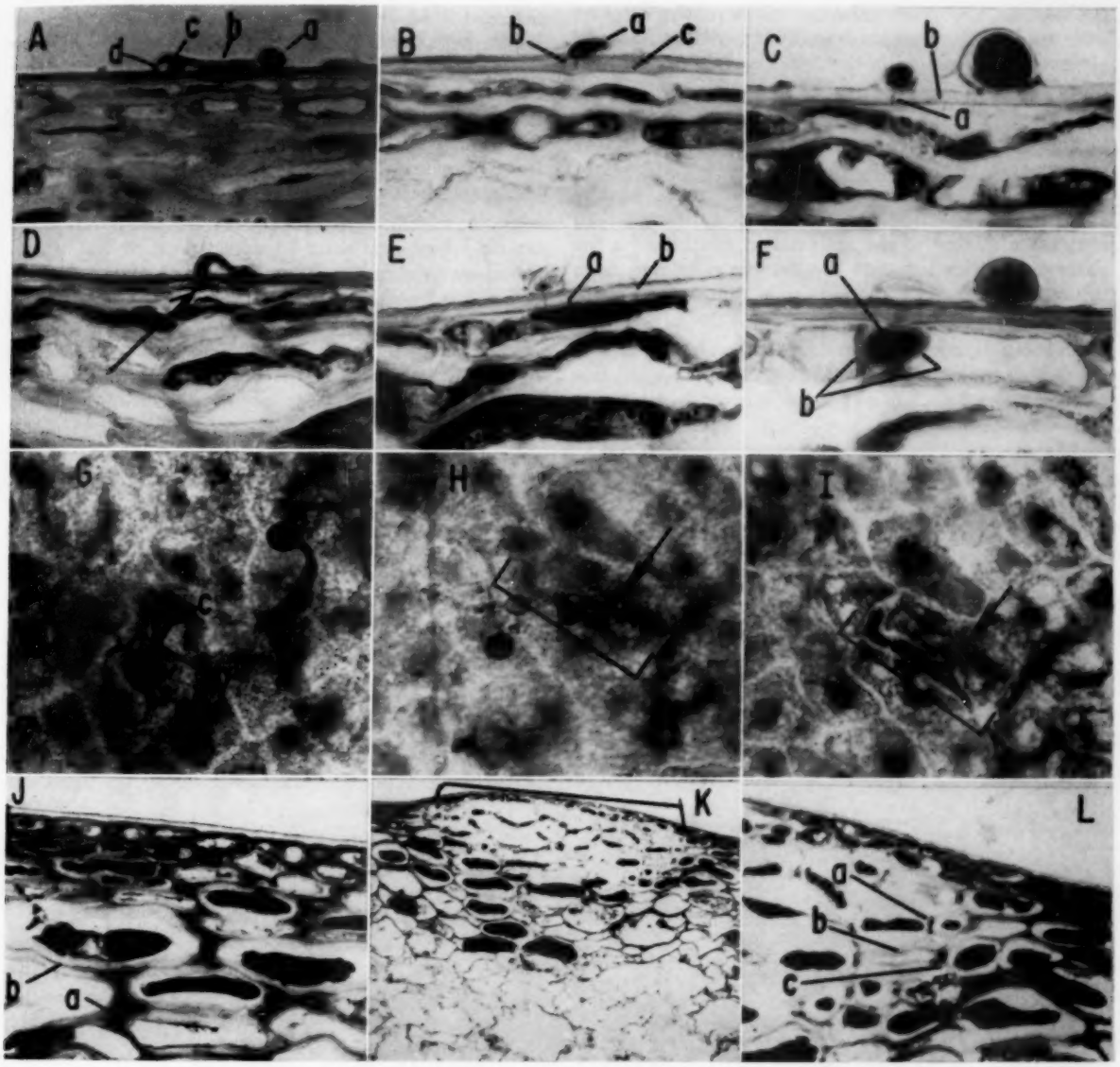

FIG. 2. A–F) Cross sections of the epidermal tissue of grapes showing stages of infection by *B. cinerea*. A) Conidium (a), germ tube (b), and appressorium (c), on the darkly stained cuticle (d). ×285. B) Appressorium (a) with infection peg (b) part way through the cuticle (c). ×666. C) Infection peg (a) has penetrated the cuticle (b). ×666. D) Subcuticular mycelium (arrow) has started to form. ×285. E) Longitudinal view of the subcuticular mycelium (a) immediately below the cuticle (b). ×590. F) Crosssectional view of the mycelium (a) beneath the cuticle and between the adjacent walls (b) of 2 epidermal cells. ×666. G-I) Views of the skin of grapes infected by *B. cinerea* as seen from above. ×333. G) Germinated conidia on the surface of the cuticle. The central conidium (a) has formed a germ tube (b) and appressorium (c). The infection peg is indicated by the densely stained spot in the center of the appressorium. The conidium at the upper right has produced 2 germ tubes, one longer than the other. H, I) Two views of the same area, one in a plane 5 μ above the cuticle, the other 5 μ below. The location of the infection peg is indicated by an arrow in each view, the subcuticular mycelium by a bracket. The coarse granular appearance of the host cells adjacent to the mycelium indicates that the protoplasm has been injured or killed by the pathogen. J-L) Cross sections of grape epidermal tissue showing the distribution of the pectic substances in the cell walls (stained dark). J) Unaffected tissue showing the relatively greater amount of these materials between the anticlinal (a) than between the periclinal walls (b). ×267. K) Low-power view of a lesion illustrating the disappearance of pectic substances in the macerated area (bracketed). ×52. L) High-power view showing some pectic material still present in and between the anticlinal walls of partly macerated cells (a). Periclinal walls nearly devoid of these substances (b). The oval to irregular dark areas are cell contents (c). ×155.

appears to be no reason why such a phenomenon could not take place. Also, in no case did a germ tube form more than 1 appressorium. Although the appressorium in Figure 2, A appears to be located in an intercalary position on the germ tube, no positive observations were made to support this possibility. All appressoria observed were located in the distal portion of the germ tube.

The subcuticular mycelium is visible as a tortuous, torulose segment between the anticlinal walls of the epidermal cells (Fig. 2, H, I). The protoplasm of the host cells adjacent to the mycelium is heavily stained and has a coarse granular appearance, in contrast with that of the cells not in contact with the pathogen, indicating that the former cells had been injured or killed by the action of the fungus prior to fixation.

Eighteen hours was the shortest incubation period for which penetration could be detected with this method. The infection peg was observed to penetrate directly through the cuticle from the appressorium to the epidermal cells below. Within the cuticle, the diameter of the peg was less than 1 μ, and between the cuticle and the epidermis it was 10 μ. In the material fixed after 24 hours, the vesicle-like invading hypha between the epidermal cells had elongated to 50 μ. After 30 hours, it had branched 1 or 2 times and appeared to have penetrated below the epidermal cells. The epidermal tissue from the 36-hour material was comparatively fragile. By this time the mycelium had branched profusely between and below the epidermal cells.

Some conidia produced very short germ tubes before approssoria were produced (Fig. 2, C), whereas others produced germ tubes up to 150 μ in length before appressoria were produced (Fig. 2, C), where- observed the same phenomenon on epidermal strips of tomato infected by *B. cinerea*. They concluded that the long germ tubes were occasioned by poor attachment to the host surface when abundant water was

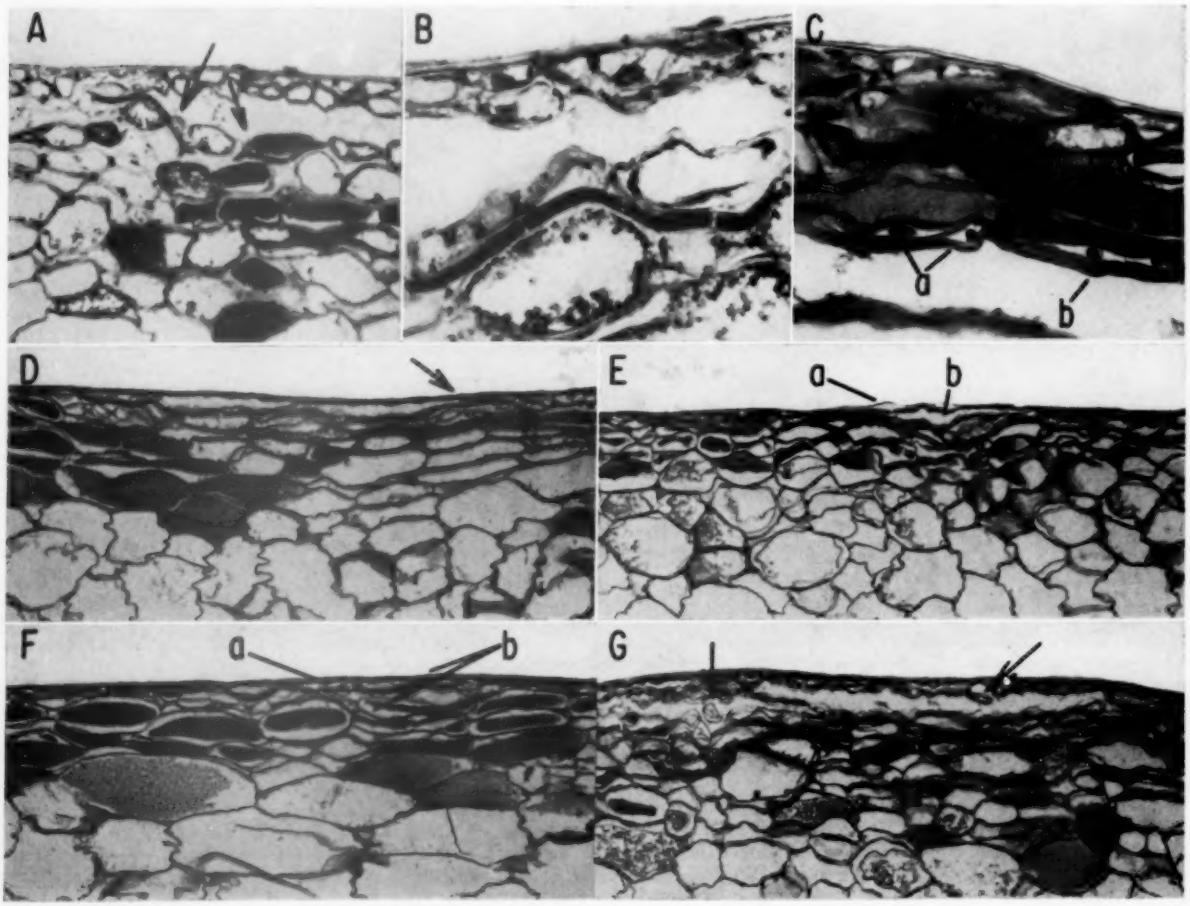

FIG. 3. Cross sections of grape epidermal tissue infected by *B. cinerea*. A-C) Affected tissue showing the relationship of the mycelium of the pathogen to the host cells. A) Two hyphal segments near the edge of a lesion (arrows). ×52. B) Detailed view of an intercellular segment. ×300. C) Segments in cross section: *a*, intercellular, *b*, intracellular. ×300. D-G) Types of tissue maceration in slipskin lesions. ×80. D) Lower periclinal walls of the epidermal layer have become separated from the adjoining walls of the subepidermal layer (arrow). E) The cuticle (a) has become separated from the epidermal layer (b). F) The periclinal walls of the subepidermal layer (a) have become separated from those above and below (b). G) Two cells have become nearly detached in the tissue (arrows).

present.

Some of the sections stained with haematoxylin were dehydrated and mounted in balsam in order to study the progressive orientation of the mycelium in relation to the host tissue after infection. In no case had the fungus penetrated deeper than 5–8 cell layers (Fig. 3, A, B), even when the lesion was 1 cm in diameter. In all sections, distinct cell-wall separation within close proximity of hyphae was evident. In only a few instances was the mycelium found within the host cells (Fig. 3, Cb).

LESION DEVELOPMENT.—Conidia for the spore suspensions were taken from infected grape clusters that had been incubated in loosely covered boxes at room temperature until the clusters were covered with a densely sporulating mat of the fungus. These clusters were held over tap water, and a slight air movement carried clouds of spores onto the water surface, where they remained and could be shaken into suspension. Healthy clusters were sprayed with this suspension, then placed in moisture-proof bags and incubated at 20°C.

After 48 hours, berries that had been almost devoid of pigment showed distinct lesions 1–4 mm in diameter (Fig. 1, F). At this stage, the skin over the lesions showed considerable loss of tensile strength. Cracks appeared spontaneously across the lesions, owing to stress induced by the turgor of the berry (Fig. 1, Fa). When moderate pressure was applied to the berries, very small cracks appeared, each marking the site of a previously invisible lesion.

Three zones can be recognized in the largest lesion on the berry in Figure 1, G. The slightly darkened outer zone is caused by separation of the epidermis from the subepidermal layers. The very dark sharply defined intermediate zone indicates the region of killing of the protoplasts of the skin cells. The innermost zone is lighter because of the air space under the epidermis, a symptom observed by Ainsworth (1) on tomatoes infected by *B. cinerea*. The pigment of the berry in Figure 1, G is more dense than that of the berry in Figure 1, F, a condition tending to mask the discoloration of the subcuticular tissue of the lesion.

A histological study of lesion development was made on tissues taken from the surface of berries that had been inoculated as previously described and incubated for various periods under moist conditions at 20°C. The lesions could first be detected as brown spots 0.25 mm in diameter on light-colored berries 36 hours after inoculation. The material was fixed in Karpechenko's solution, washed, dehydrated, and infiltrated with paraffin (14). The sections were cut 25 μ in thickness, exposed to 10 per cent tannic acid mordant for 30 minutes, then stained with methylene blue as described by Ridgway (15).

A few cells of skin tissue had separated after 36 hours (Fig. 4, A). After 42 hours of incubation, cell separation had progressed laterally, and the protoplasts of some cells had collapsed (Fig. 4, B). After 48 hours, cell separation was still confined to the outermost 3 or 4 cell layers of the epidermal and first subepidermal layers (Fig. 4, C, D). After 60 hours, most of the cells exterior to the pulp had died (Fig. 4, E). A large air cavity was visible between the remnants of the subepidermal cells and the epidermal layer still attached to the cuticle.

Sections for wall-separation studies were cut 10 μ in thickness and treated according to the abbreviated haematoxylin schedule described by Esau (7). The lower periclinal walls of the epidermal cells usually become separated from the upper walls of the adjoining subepidermal layer (Fig. 3, D). The epidermis and cuticle remain relatively intact over the lesion. In a few cases, the cuticle was separated from the epidermal cells (Fig. 3, E). The periclinal walls of the first subepidermal cell layer frequently become separated from those of the layers both above and below, whereas the anticlinal walls remain together (Fig. 3, F). As a result, the affected tissue tends to slip easily in shallow horizontal planes when subjected to lateral pressure. Individual cells often become nearly detached from the adjacent cells (Fig. 3, G). In this figure, 2 layers of skin cells are detached from the underlying tissue, a condition less frequently observed than that shown by Figure 3, D. Only occasionally were the upper 3 cell layers separated as a distinct unit, and very seldom 4.

ENZYMATIC ACTION.—From germinated spores of *B. cinerea* growing in turnip juice, Brown (3) prepared an extract that caused maceration of thin slices of turnip and carrot tissue *in vitro*. In later studies (4), a similar extract had no effect on the cuticle of bean leaves or of flower petals, but when introduced into the subcuticular tissue, it caused rapid disorganization. Weimer and Harter (16) designated as pectinase the enzyme, produced by *B. cinerea*, that dissolved the middle lamella of discs of sweet potato tissue.

An extract of germinated Botrytis spores, prepared as described by Brown (3), caused maceration of thin slices of grape tissue. Sections cut 1 mm thick and perpendicular to the surface were placed in the extract and held at room temperature. Three hours later, some maceration was evident when the material was teased apart with dissecting needles. After 6 hours, the skin tissue could be separated easily from the pulp, after which it disintegrated into distinct sheets of periclinal layers 1-cell thick. Maceration was of the type shown in Figure 4. Extract that had been heated to 100°C for 5 minutes had no macerating effect.

Some of the sections prepared for penetration studies were treated with the spore extract. The previously described staining schedule was used, except that an aqueous 1:5000 stain of ruthenium red for 10 minutes (13) was included. In the region 3–8 cell layers below the cuticle, the zone of the pectic substances is very thin and sharply defined between the periclinal walls of unaffected tissue (Fig. 2, J). It appears much thicker in the walls of the epidermal and subepidermal layers and in the anticlinal walls

of cells further removed from the cuticle. It appears that pectic materials permeate the cellulose of these walls in addition to being present between the walls. In one area (a) between the anticlinal walls, the material of the wide zone is sufficiently diffuse to make the narrow intercellular zone visible.

In Figure 2, K the pectic materials in the affected area are less dense than those in the unaffected tissue on each side of the lesion, indicating that the fungus has caused the disappearance of the pectic materials.

The type of maceration shown in Figure 4, K, L is very similar to that shown in Figure 3, F. The intercellular material between the anticlinal walls apparently has been less subject to dissolution than that between the periclinal walls, resulting in separated periclinal layers of cells in the lesion. The darkly stained large areas in Figure 3, Lc indicate the location of cell contents, whereas the smaller vertical "bars" (Fig. 3, La) indicate the location of the anticlinal walls, which in this case were perpendicular to the plane of sectioning. The periclinal walls were only faintly visible (Fig. 3, Lb), presumably because of the dissolution of the associated pectic materials.

Discussion.—*B. cinerea* is the only common decay organism that produces the type of lesion on grapes described in this paper. Berries infected by *Penicillium* sp, *Rhizpous nigricans*, and *Aspergillus niger*, for example, appear to be macerated uniformly through-

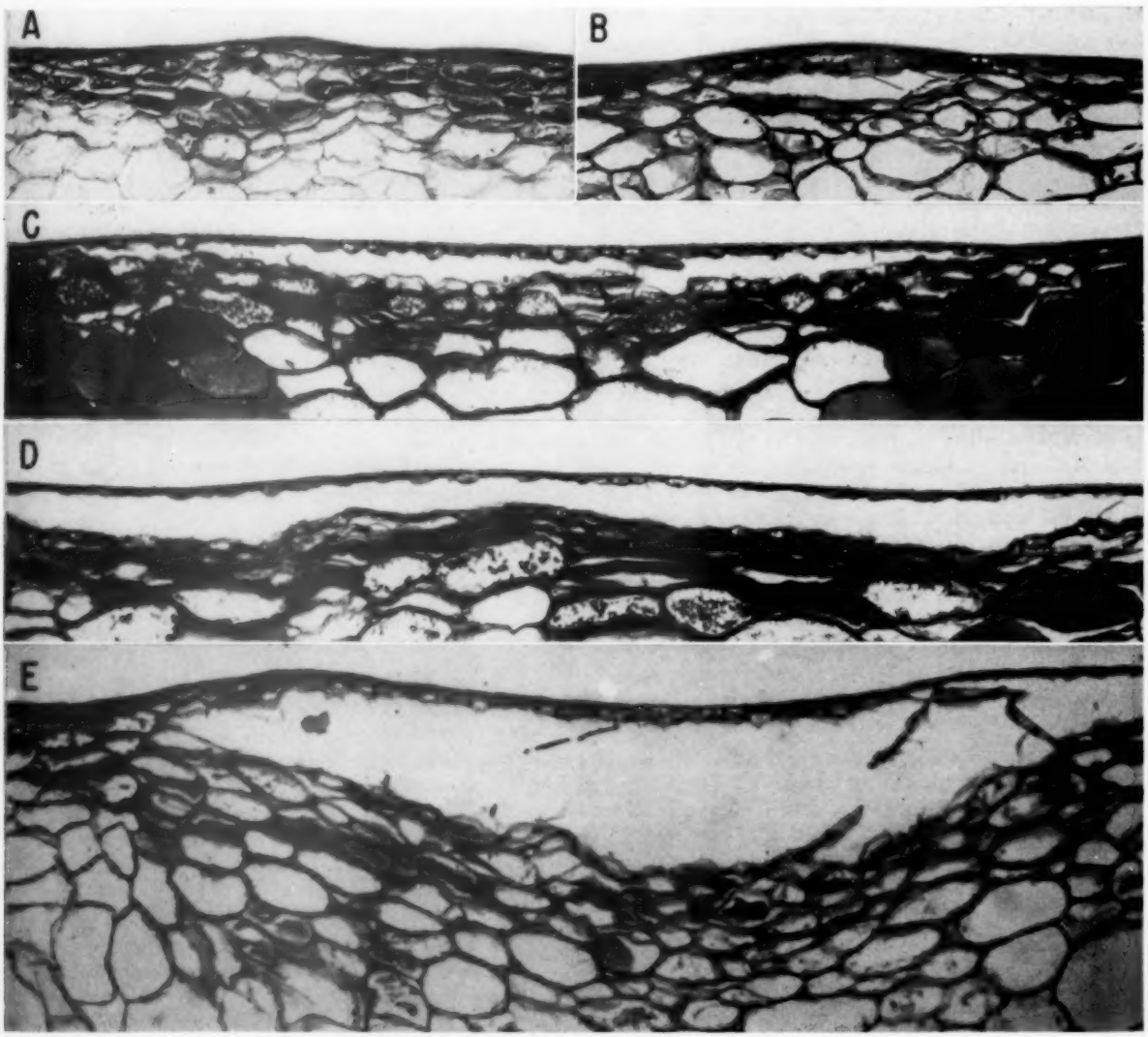

Fig. 4. Cross sections of grape slipskin lesions following inoculation and incubation at 20°C. ×80. A) After 36 hours. First macroscopically visible lesion ¼ mm in diameter. A few cells of the skin tissue have become separated. B) After 42 hours. Separation of the skin cells has progressed laterally and the protoplasts of some cells have collapsed. C, D) After 48 hours. Maceration involves only the outermost 3–4 cell layers, and lateral progress is variable. Only the cuticle and epidermis, D, or the cuticle, epidermis, and remnants of the first subepidermal cell layer, C, are continuous over the lesion. E) After 60 hours. Maceration has affected cells nearly to the pulp, resulting in a considerable air cavity under the epidermis.

out. It is doubtful that this distinct difference in type of lesion can be explained satisfactorily on a basis of differences in the regularity of cell orientation within the tissue or of differences in distribution of intercellular material between the anticlinal walls and between the periclinal walls. A more logical explanation may be that there are differences in the chemical composition of the pectic materials in the different tissues. If some constituents of the pectic materials in and between the anticlinal walls of the epidermal tissue and between all of the walls of the pulp tissue are relatively unaffected by the hydrolytic enzyme system of *B. cinerea*, the peculiar type of lesion that develops when grapes are attacked by this organism could be explained. The enzyme systems of other decay organisms may be able to hydrolyze all pectic materials, including those not affected by the enzymes of *B. cinerea*. That the pectic material in different tissues may differ is indicated by the observation of Rawlins (13) that pectose between the lamellae of cellulose walls appears to be less resistant to acid hydrolysis than the pectic materials of the middle lamella.

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A DISEASE OF BEAN INCITED BY HELMINTHOSPORIUM VICTORIAE[1]

N. N. Winstead and T. T. Hebert

SUMMARY

A species of *Helminthosporium* has been found to cause a hitherto unreported disease of bean in southeastern North Carolina. Lesions were found principally on bean pods but also were observed on stems, petioles, and veins of leaves. Small water-soaked spots, approximately 1 mm in diameter and with black necrotic centers, formed on the pods. Lesions on stems, petioles, and veins appeared as small, narrow, dark brown to black streaks. The disease has been observed in the field only when beans were grown adjacent to fields of oats heavily infected with *H. victoriae*. The Helminthosporium pathogen isolated from bean was found to be identical morphologically and pathogenically with the Victoria blight incitant, *H. victoriae*.

In late May, 1954, marketable-size green pods of the Tendergreen variety of bean, *Phaseolus vulgaris* L., that showed unusual disease symptoms were collected from a field in Pender County, North Carolina. Although lesions appeared to be superficial, almost all the pods were spotted with numerous lesions of a type not heretofore observed on this crop. Subsequently, infections on leaves, stems, and petioles were observed and isolations from all diseased plant parts consis-

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tently yielded a species of *Helminthosporium*. The disease also was found in the field on the bean varieties Black Valentine, Bountiful, Contender, Tenderlong 15, and Wade. Diseased beans were found in a 60-acre field adjacent to Victorgrain oats that were severely affected with the Victoria blight disease incited by *Helminthosporium victoriae* Meehan & Murphy. Infected bean pods were observed throughout the field; however, infections were most severe in the areas adjacent to the oat field. Futhermore, no new infections on bean were observed after the oats were harvested and the stubble plowed under. This observation suggested an association between the diseases on the 2 crops.

No report was found in the literature of a species of *Helminthosporium* parasitic on bean. Meehan and Murphy[2] reported that *H. victoriae* occurs as a saprophyte or weak parasite on soybean, *Glycine max* (L.) Merrill.

The symptoms of the disease on bean and results of studies on the relationship of the disease on oats and bean are reported herein.

Symptoms.—The lesions were more obvious and numerous on pods than on stems, petioles, or leaves. On pods, the first symptoms appeared 48–72 hours after inoculation as small water-soaked spots approximately 1 mm in diameter and with indefinite margins. Shortly after the water-soaked areas appeared, small black spots, approximately ½ mm in diameter, were formed in the center of the lesions (Fig. 1), and occasionally narrow black elongated lesions were formed along the sutures of the pods. In the field, 40–50 spots frequently were counted on pods not covered by foliage. Few spots developed on pods or portions of pods that were protected by foliage. Leaf symptoms appeared primarily on the veins of the lower surfaces. Small, dark brown to black, narrow streaks from 1 to 5 mm in length formed on both naturally and artificially inoculated plants. In addition, small dark spots were sometimes observed on the blades of expanded leaves. Stem and petiole symptoms were similar to those on the veins of leaves.

No fungus spores were observed in specimens collected in the field; however, when infected pods were placed in a moist chamber for a week, very sparse sporulation occurred.

Causal organism.—Isolations from pods, stems, petioles, and leaves consistently yielded a species of *Helminthosporium* that appeared to be quite similar to *H. victoriae* Meehan & Murphy. Measurements were made of 25 conidia from each of 3 isolates from bean grown on potato-dextrose agar. The spore measurements were as follows: length, 37–73 μ (mean, 48.4 μ); width, 10–16 μ (mean, 13.3 μ); and number of septa, 3–8 (mean, 5.6). Only slight differences in size were found among the 3 isolates. The spore measurements were within the range described for *H. victoriae*.[2,3]

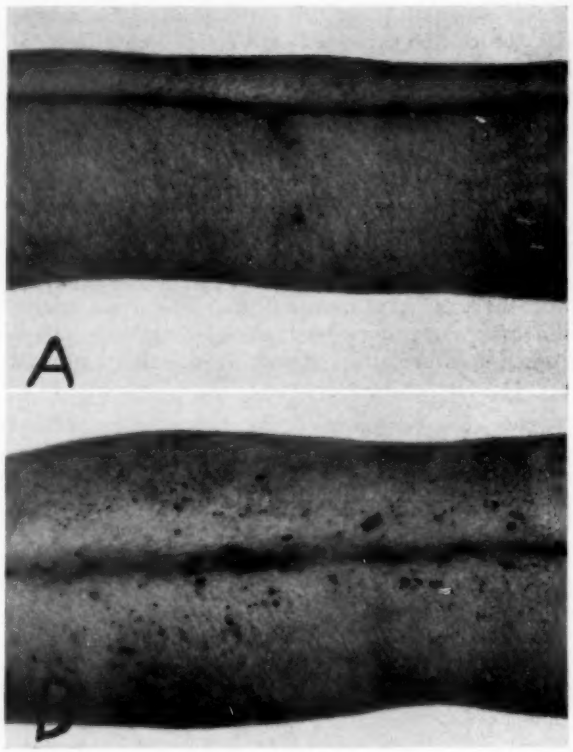

Fig. 1. Bean pods infected with *H. victoriae*. A) Young pod 5 days after inoculation. B) Old pod 2 weeks after inoculation.

Pathogenicity studies.—Fifty seeds each of the Victoria blight susceptible oat varieties Arlington and Victorgrain and of the resistant varieties Fulghum and Lee[4,5] were soaked for 30 minutes in aqueous suspensions of hyphae and spores of 2 bean isolates, then placed between moist paper towels. In 3 tests, only 6 inoculated seeds of the susceptible varieties germinated, and these plants died in the seedling stage. Germination of seeds of the inoculated resistant varieties and of noninoculated susceptible and resistant varieties was 90 per cent or higher, and no symptoms of blight developed on these seedlings. Oat plants of these 4 varieties, grown in 4-in. clay pots in the greenhouse, were inoculated by spraying the host material with an aqueous suspension of hyphae and spores just after emergence, when 4 in. in height, or when 8 in. in height. Approximately 50 plants of each size and variety were used. Symptoms of Victoria blight were produced only on plants of the Victorgrain and Arlington varieties.

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[3] Lutrell, E. S. 1955. A taxonomic revision of Helminthosporium sativum and related species. Amer. Jour. Bot. 42: 57-68.

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[5] Wells, J. C., and T. T. Hebert. 1951. Small grain diseases. N. C. Agr. Ext. Circ. 347, 32 p.

Twenty plants each of the Wade and Contender bean varieties, bearing pods ranging from ¼ in. to marketable size, were inoculated by spraying with an aqueous suspension of hyphae and spores. Lesions were numerous on pods that were of marketable size when inoculated, whereas few lesions formed on pods that were less than 3 in. in length at the time of inoculation. Leaf symptoms in greenhouse tests were more severe than those observed in the field. Isolations from lesions of either host consistently yielded *H. victoriae*.

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PHYTOPATHOLOGICAL NOTES

A Petal Blight of Chrysanthemum Incited by Itersonilia perplexans.[1] LOUISE T. DOSDALL. In November, 1951, *Itersonilia perplexans* Derx was isolated from blighted pompom type Chrysanthemum blossoms growing in a local commercial greenhouse. The basis for identification of the fungus, results of pathogenicity studies, and a description of affected blossoms of Chrysanthemum and snapdragon varieties are presented herein.

This blight was somewhat different from the common Botrytis blight. The tip halves of the outer petals (about ¼ of the petals in each blossom) were brown and dried. The blight was very severe on the variety Marketeer (pink), moderately severe on Helen Frick (white), and light on Friendly Rival and Gladys Pierson. The grower reported that the blight had developed suddenly during several days of warm weather while the greenhouse was closed tightly. After the ventilators were opened, no further development of the disease was noted.

The diseased tissue was filled with broad hyphae with prominent clamp connections at the septa. In a moist chamber, the hyphae produced characteristic sterigma-like conidiophores with asymmetric ballistospores. The fungus was easily isolated by suspending pieces of the brown petals on the inside of the covers of petri dishes over potato-dextrose agar,[2] malt agar,[3] or 1.5 per cent Pablum[4] agar. Within 48 hours, numerous colonies appeared on the agar directly under the pieces of diseased tissue. The hyphae in these colonies had prominent clamp connections at the septa; produced chlamydospore-like structures, each with a prominent clamp connection at its base; and produced characteristic asymmetric ballistospores. No fungus colonies with clamp connections developed on the agar under the pieces of tissue from the healthy lower half of the blighted petals, from nonblighted petals, or from healthy green leaves. The first leaf below the blossom was used for these tests. Mirror-image colonies were obtained easily from some of these original colonies by inverting the plates over fresh media. Although no fungi with clamp connections were obtained from healthy leaf tissue, 5 colonies of Sporobolomyces and 16 colonies of Bullera were obtained on 1 plate. No colonies developed under the healthy petal tissue.

In tests on the pathogenicity of the fungus isolated from Chrysanthemum petals, 2 white pompom blossoms with about 8-in. stems were placed in a tall jar with about 2 in. of water in the bottom, and a petri dish culture of the fungus was suspended over the top. The whole was covered by a glass bell jar. Two days later, there were no obvious indications of infection. When the epidermis of one of the petals was examined under the microscope, many ballistospores were found on the surface, but only an occasional spore had germinated. In no case could penetration of the petal tissue be ascertained. On the fifth day, however, the tip halves of about 20 petals on each blossom were brown, many more petals had tiny brown spots, and tiny red spots, each involving about 6 host cells, were found by means of a microscope in the petal tissue that was still white. Each such spot contained a broad intercellular hypha. In the brown tissue of the petals, the host cells had collapsed and contained many hyphae with prominent clamp connections at the septa. Also present were many chlamydospore-like bodies, each with a conspicuous clamp connection at the base, and innumerable ballistospores. Brown irregular blotches 1–2 cm in diameter also had developed on the leaves.

Itersonilia was reisolated by suspending pieces of the following kinds of tissue over malt agar in petri dishes: 1) blighted petal tips, 2) petals with tiny red spots, 3) white healthy-appearing petals, 4) leaf lesions, and 5) healthy-appearing green leaves. Two

[1] Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota. Paper No. 3437, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] BVL Potato Dextrose Agar Tablets distributed by Ben Venue Laboratories, Inc., Bedford, Ohio, plus 0.3 per cent yeast extract (Difco Laboratories, Detroit, Michigan).

[3] BVL Malt Dextrose Agar Tablets plus 0.3 per cent yeast extract (Difco).

[4] Pablum is a baby food manufactured by Mead Johnson & Co., Evansville, Ind. (Contains wheat meal, oatmeal, wheat germ, cornmeal, bonemeal, sodium chloride, alfalfa meal, brewers' yeast, iron).

days later, colonies of Itersonilia had developed under the pieces of blighted petal tissue and under the brown leaf tissue. After 4 days, colonies developed under the petal tissue with tiny red spots. After 10 days, colonies of Itersonilia had developed under 1 of the 5 pieces of apparently healthy white petal tissue, and under 1 of the 5 pieces of green leaf tissue.

Similar inoculations were repeated on other Chrysanthemum blossoms from the same source. The results were similar.

Inoculations were made also using cut blossoms of 3 varieties of hardy garden Chrysanthemums: Sun Red, an unnamed seedling with yellow anemone-type blossoms. The petals of the flowers under suspended Itersonilia cultures became blighted on each of the 3 varieties whereas those in the check jars did not. Leaf lesions developed only on the variety Sun Red. Itersonilia hyphae and ballistospores were abundant in the diseased tissue.

White snapdragon blossoms were inoculated in the same manner and developed a similar blight, except that they collapsed very quickly and fell from the stems. Itersonilia hyphae were found in the tissues.

The ballistospores of the fungus from Chrysanthemum measured from 10 to 17 μ in length and from 7 to 10 μ in width. These measurements, the broad hyphae with conspicuous clamp connections at the septa, and the chlamydospore-like bodies with a conspicuous clamp connection at the base of each agreed in all respects with the description of the fungus isolated by Derx[5] from suspended leaf tissue of *Althaea rosea* (L.) Cav. infected with *Puccinia malvacearum* Mont. and with that of the fungus isolated by Tubaki[6] from the leaves of 8 different kinds of plants in Japan. In 1948, Derx described his fungus as a new species of a new genus, *Itersonilia perplexans*. In 1949, Nyland[7] described a second species of Itersonilia, *I. pyriformis*, which he isolated from a dead leaf of *Acer macrophyllum* Pursh in Washington state. Tubaki, as well as Olive,[8] considers Nyland's fungus to be a variant of *I. perlexans*. In 1952, Wilkinson[9] reported a species of Itersonilia as causing a blight of the leaves and a dry rot of the roots of parsnips in the eastern United States and in England, and in 1953, Sowell[10] described the penetration of parsnip leaf tissue by the same fungus. Recently, Robertson[11] reported a blossom scorch on more than 70 varieties of Chrysanthemum in many nurseries in England. His illustrations of a diseased blossom, his "sporidia" measurements, and his description of mycelia agree well with those given here. He suggested that the fungus resembles *Entyloma calendulae* (Oudem.) d By. in some respects although no infection was obtained on Calendula. A detailed study of its identity has not yet been reported.

I. perplexans and/or its variants seem to be widely distributed, having been isolated from a wide variety of plants in the Philippines[5], Japan[6], Washington[7], Louisiana[8], New York[9], Minnesota, and probably England[9, 11]. Further work is needed on the life history and taxonomic relationships of the fungus, on the occurrence of the fungus on both healthy and diseased plants, and on the pathogenic or saprophytic nature of various isolates. Some of the investigations on *Entyloma* species, Taphrina, and possibly Exobasidium should be re-evaluated and reinvestigated.—Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

The Age of Cultures of Xanthomonas pruni in Relation to Infectivity. ANGELOS V. ADAM AND DWIGHT POWELL.[1] During the course of studying the epidemiology of *Xanthomonas pruni* (E. F. Sm.) Dows., it was necessary to determine the age at which bacterial cultures would produce maximum bacterial spot infection on peach trees by artificial inoculation. No report could be found in the literature covering this subject.

A block of 3-year-old Elberta peach trees was divided into single-tree plots with each plot replicated 4 times. A thorough examination of these trees revealed no evident infection. The inoculum was grown and applied according to the method described by Adam et al.[2] Emerson-broth cultures were prepared and incubated for 6, 12, 18, 24, 36, 48, 72, 144, 288, and 576 hours. These cultures were made by transferring the inoculum at appropriate time intervals so that all would reach the end of the desired incubation period at a certain predetermined time (August 18, 10 a.m.). All inoculations were made on this date in 1953. Ten days later (August 28), counts of leaf infection were made, and the next spring (May 28, 1954), all plots were checked for the presence of spring cankers.

The degree of infectivity, as manifested by both kinds of counts, increased progressively and reached a maximum after 48 hours of incubation (Table 1).

[5] Derx, H. G. 1948. Itersonilia, Nouveau genre de Sporobolomycetes a mycelium boucle. Buitenzorg Jard. Bot. Bul. Ser. 3, 17: 465-472.

[6] Tubaki, K. 1952. Studies on the Sporobolomycetaceae in Japan. II. On Itersonilia. Nagaoa (Mycol. Jour. Nagaoa Inst. Tokyo) No. 2. p. 62-66.

[7] Nyland, G. 1949. Studies on some unusual Heterobasidiomycetes from Washington state. Mycologia 41: 687-701.

[8] Olive, L. S. 1952. Studies on the morphology and cytology of Itersonilia perplexans Derx. Torrey Bot. Club Bul. 79: 126-138.

[9] Wilkinson, R. E. 1952. Parsnip canker is caused by Itersonilia sp. (Abs.) Phytopathology 42: 23.

[10] Sowell, G., Jr. 1953. Infection of parsnip by Itersonilia sp. (Abs.) Phytopathology 43: 485.

[11] Robertson, A. G. 1955. Flower scorch of Chrysanthemum. Plant Pathology (Harpenden, Eng.) 4: 33.

[1] Assistant and Professor of Plant Pathology, respectively.

[2] Adam, A. V., D. Powell, and H. W. Anderson. 1955. Time of peach twig infection by Xanthomonas pruni in relation to spring-canker incidence. Phytopathology 45: 285-287.

TABLE 1.—*Numbers of infections produced on peach by cultures incubated for different periods*

Age of culture in hours	Average number of infected leaves per tree, August 28, 1953	Average number of cankers per tree May 28, 1954
6	1.5	0.7
12	6.2	1.0
18	12.5	3.7
24	26.0	6.0
36	38.8	11.2
48	43.0	11.7
72	32.0	6.0
144	19.0	2.5
288	11.0	1.5
576	8.5	0.5
Water check	0.2	0.7
LSD at 1 per cent level	10.7	4.02

This conforms closely to cell counts from plate cultures, which indicated that the highest number of active bacteria was obtained after 72 hours of incubation. Cultures grown for 48 to 576 hours produced progressively less infection. — Department of Plant Pathology, University of Illinois and Illinois Agricultural Experiment Station, Urbana, Illinois.

Mechanical Transmission of Sweetpotato Internal Cork Virus Aided by Cysteine. E. M. HILDEBRAND. After the discovery that rapid-growing Scarlett O'Hara morning glory is an ideal indexing host for sweetpotato internal cork virosis,[1] it still was not possible to study the nature and properties of the causal virus. Apparently the virus in the extract from infected sweetpotato plants is inactivated so quickly upon contact with air that the usual mechanical methods of transmission are unsuccessful. The success of a modification of Yarwood's[2] inoculation procedure in locating Scarlett O'Hara morning glory as an indexing host was attributed[3] to the extremely short interval of virus exposure (about $\frac{2}{3}$ second) by this "squeeze-rubbing" technique.

An examination of the literature was relatively fruitless in finding a comparable host-virus situation. Bald and Samuel[4] found that of 6 reducing chemicals tested, only sodium sulphite markedly reduced inactivation of tomato spotted wilt virus. Four of the chemicals, including cysteine hydrochloride, hastened inactivation. Subsequently, Best and Samuel[5] found that when the reaction of the virus extract was controlled at pH7, cysteine had a notable preservative action on tomato spotted wilt virus. This report is concerned primarily with the remarkable preservative or protective action of cysteine on the internal cork virus.

Exploratory tests were made with nonreducing substances—water, phosphate buffer (K_2HPO_4, 1 per cent), sucrose,[6] and egg albumen[7]—all with indifferent results. The most readily accessible reducing chemicals — cysteine, sodium sulfite, sodium hyposulfite, and sodium monosulfide — were tested. Of these, cysteine and sodium sulfite best protect the sweetpotato internal cork virus from rapid inactivation, and cysteine apparently makes it most readily transmissible by mechanical means. When root or leaf tissues of diseased sweetpotato plants were ground in the presence of 0.08 M cysteine and rubbed on morning glory leaves that had been moistened with 1 per cent K_2HPO_4 buffer and dusted with Carborundum, positive vein-banding mottle symptoms appeared within 9 days (Fig. 1). The "tissue squeeze" rubbing technique[1] was a very reliable indexing method for checking on the cysteine technique.

This stabilizing chemical has made possible preliminary observations on several properties of the virus. Active virus was present in sediment from centrifuging at 2000, 8000, and 20,000 r.p.m. The dilution endpoint was approached when the volume of the tissue extract was approximately equal to that of the cysteine solution. That is, infectivity appears lost at dilutions slightly above 1:2 but still under 1:10. At greenhouse temperatures of about 30°C, the virus extracts containing 1.0, 0.1, or 0.01 per cent cysteine retained activity for a maximum of 3 hours, whereas several preparations held at 6°C were still active after 26 hours. In 1 series of tests, a mixture of equal volumes of 1 per cent cysteine and virus extract was active after a 1-minute exposure at 60°C but was inactive after a 2-minute exposure. The virus survived 5 min-

[1] Hildebrand, E. M. 1956. Sweetpotato internal cork virosis indexed on Scarlett O'Hara morning glory. Science 123: 506-507.

[2] Yarwood, C. E. 1953. Quick virus inoculation by rubbing with fresh leaf discs. U. S. Dept. Agr., Pl. Dis. Rptr. 37: 501-502.

[3] Hildebrand, E. M. 1943. Micrurgy and botany with special reference to phytopathology. In Reyniers, J. A., ed., Micrurgical and germ-free techniques. Charles C. Thomas, Springfield, Ill. p. 72-92.

On page 81, the writer reported a mechanism by which viruses may gain entry into plant cells: ". . . the phenomenon of momentary protrusion of protoplasm through a perforation in the wall of an injured cell. . . . When the micropipette perforates the trichome wall, protoplasm gushes out to form a hemispherical droplet which bathes the pipette tip momentarily and then returns into the cell. . . . It is rather apparent that since a similar reaction follows the perforation of epidermal cells, other than hairs, this phenomenon may be rather general in plants."

[4] Bald, J. G., and G. Samuel. 1934. Some factors affecting the inactivation rate of the virus of tomato spotted wilt. Ann. Appl. Biol. 21: 179-180.

[5] Best, R. J., and G. Samuel. 1936. The effect of various chemical treatments on the activity of the viruses of tomato spotted wilt and tobacco mosaic. Ann. Appl. Biol. 23: 759-780.

[6] Hansen, H. P. 1954. The influence of saccharose on infectivity and physical properties of some plant viruses. [Denmark] Roy. Vet. and Agr. Col., Dept. of Pl. Path. Contrib. 39, 18 p.

[7] Finlay, K. W., and C. A. Parker. 1954. The preservation of tomato spotted wilt virus by vacuum drying. Austral. Inst. Agri. Sci. Jour. 20: 112-114.

FIG. 1. Vein-banding mottle symptoms on Scarlett O'Hara morning glory 7 days after mechanical inoculation with mixture of extract from corky roots of sweetpotato and 1 per cent cysteine, in equal amounts. Before inoculation the plant was pruned to a single leaf. Eight of the new leaves that unfolded after inoculation are showing symptoms.

utes at 55°C but was inactivated in 10 minutes. In an inactivation experiment involving the immersion of diseased tissues, in hot water, the thermal inactivation point was 56°C for the petiole (the largest in size), 55°C for the root, and 54° for the leaf. Thus the virus apparently has a thermal inactivation point above 55°C.—Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland.

A Vacuum Drying Process for Preservation of Puccinia graminis.[1] DONALD M. STEWART.[2] The preservation of viable rust cultures at the Cooperative Rust Laboratory, St. Paul, Minnesota, requires the maintenance of as many as 1000 uredial isolates, representing 100 or more physiologic races. Viability of cultures of rust fungi is maintained by passage through the host plant about every 4 months, and spores are stored in glass vials under refrigeration in the intervals. Maintenance of cultures in this way involves considerable labor, risk of contamination, and occasional loss of important material because of mechanical failure in the refrigeration unit. Obviously, it would be desirable to be able to store cultures a year or more without passage through the host.

The lyophilization technic that has been widely used for bacteria[3] comprises freezing in some type of suspending medium such as blood serum or a solution of gelatin or sucrose and subsequent sublimation of water from the preparation at reduced pressure. This same process was also applied to cultures of fungi by Raper and Alexander.[4] More recently Sharp and Smith[5] used it in attempts to preserve urediospores of *Puccinia coronata* Corda and of *P. graminis* Pers. var. *avenae* (Eriks. & E. Henn.) Seym. without measurable survival. These same workers developed a satisfactory method to lyophilize urediospores by drying under vacuum at room temperature and subsequently storing under refrigeration. A modification of this latter method was tried by the writer.

Five mg of fresh urediospores of races 7 and 8 of *P. graminis* Pers. var. *avenae* (Eriks. & E. Henn.) Seym., 15B and 56 of *P. graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Shear et al. and of *P. graminis* Pers. var. *secalis* (Eriks. & E. Henn.) Seym. were placed separately in 8-mm Pyrex vials. Nothing was added to 1 set of 2 vials. One of the following substances was added to each of the other 4 sets: recrystallized hemin, bovine albumin powder, plain unflavored gelatin powder (Knox), and granular casein. The additives were mixed with the spores by stirring with a spatula and by whirling the vials. All vials were then dried for 30 minutes under vacuum equivalent to 3 in. of mercury, after which they were sealed and kept at room temperature (50–95°F) for 300 days. As a preliminary check between the vacuum-drying method and storage in the refrigerator, the same isolate of race 7, *P. graminis* var. *avenae*, that was used for the vacuum experiment was stored at 12°C in a cork-sealed vial for 234 days. Approximately 10 mg of urediospores from the storage vial were divided into equal parts for inoculation of Bond oats. Hemin was mixed with one half of the inoculum and nothing was added to the other half.

In all tests, spores survived best in a mixture of hemin (Table 1). When casein was added, spores of race 56 survived well but those of the other races did not. In the mixture with albumin, only spores of race 15B survived well. Spores survived poorly in mixture with gelatin. Three pots of Bond oats were inoculated with urediospores of *P. graminis* var. *avenae*,

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station. Cooperative investigations of the Plant Pest Control Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.

[2] Pathologist, Plant Pest Control Branch, Agricultural Research Service, U. S. Department of Agriculture.

[3] Flosdorf, E. W. 1949. Freeze drying. Reinhold Publ. Corp. New York. 280 p.

[4] Raper, K. B., and D. F. Alexander. 1945. Preservation of molds by the lyophil process. Mycologia 37: 499-525.

[5] Sharp, E. L., and F. G. Smith. 1952. Preservation of Puccinia uredospores by lyophilization. Phytopathology 42: 263-264.

TABLE 1.—*Results of inoculating[a] Little Club wheat, Bond oats, and Emerald rye with urediospores of P. graminis 300 days after they had been dried in vacuum and mixed with different additives*

Additive	Average percentage of plants infected by indicated fungus and number of pustules per plant[b]				
	P. graminis var. *tritici*		*P. graminis* var. *avenae*		
	Race 15B	Race 56[c]	Race 7	Race 8	*P. graminis* var. *secalis*[c]
Hemin	90–4	94–6	25–1	90–3	28–2
Albumin	36–1	0–0	4–1	2–1	0–0
Gelatin	6–1	8–1	0–0	4–1	0–0
Casein	4–1	56–6	6–1	13–1	7–1
Check (no additive)	0–0	6–2	4–1	0–0	0–0

[a] Inoculations were made from individual vials, with 2 vials in each treatment and 4 vials with nothing added as checks.

[b] The first number of each pair indicates percentage of plants infected and the second the number of pustules per plant. The number of plants per pot in each treatment varied but averaged about 20.

[c] Owing to mechanical failure in sealing vials, one half the number of vials per treatment were used for inoculations.

race 7, kept in a refrigerator for 234 days, and hemin was added after storage. Similar tests were made with spores kept under similar conditions without an additive. In the 3 trials with spores in hemin, the percentages of plants infected ranged from an average of 67 to one of 80, with an over-all average of 72 per cent. The range for the checks was 13 to 30, with an average of 19 per cent and 1 or 2 pustules per leaf.

With the isolates of *P. graminis* var. *avenae* and *P. graminis* var. *tritici* used in this study, there was no indication of a deviation in the infection types described by Stakman et al[6,7] on differential varieties as a result of vacuum drying.

Although the results of this study are preliminary, indications are that urediospores of *P. graminis* can be preserved longer when certain additives, especially hemin, are mixed with the spores prior to vacuum drying. It appears that hemin may also stimulate spore germination, as spores kept in the refrigerator and subsequently mixed with hemin caused more infection than those to which hemin had not been added.

It has been suggested by Shu, Tanner, and Ledingham[8] that a cytochrome enzyme is operative in the respiration of urediospores of the wheat stem rust fungus because spores contain hematin and react positively to the Nadi reagent.[9] Hemin mixed with urediospores may stimulate dormant respiratory mechanisms in the presence of molecular oxygen and thus stimulate spore germination.—Minnesota Agricultural Experiment Station, St. Paul.

A Method for Eliminating the Red-Stele Fungus from Valuable Strawberry Stocks. EDWARD K. VAUGHAN.[1] In any program of breeding strawberries (*Fragaria* spp.) for resistance to the red-stele disease (Caused by *Phytophthora fragariae* Hickman), the new hybrid stocks are necessarily tested in infested soils. As a result, desirable resistant lines, which may be sent to growers for further testing or which have been given names and released to commercial growers, are likely to carry the red-stele fungus either in or on the roots although they may not show symptoms when distributed. Such chance dissemination of the causal fungus would be of no great concern if all strawberry varieties grown in an area were resistant to the disease and if there were not distinct physiologic races of the fungus in different areas. Since much of the commercial crop of strawberries is still produced from varieties that are susceptible to most of the known races of the fungus, it is important that foundation stocks, free from the pathogen, be developed and maintained and that only fungus-free plants be released to growers or shipped from one area to another.

In addition to the resistant strains, it is also frequently desirable to maintain small lots of susceptible lines that are free from red-stele. These are necessary both for crossing with resistant lines and for release as foundation or mother stocks to growers of certified plants. A method of propagating strawberry plants has been used successfully at the Oregon Experiment Station for 2 seasons. It is based on 3 established principles: 1) the red-stele fungus does not invade crowns and stolons, even in susceptible varieties; 2) the fungus grows poorly when the temperature of the soil is above 65°F, and even in infested soils, susceptible varieties of strawberries will grow well during warm summer weather; and 3) the fungus does not thrive in adequately drained soil.

Plants to be propagated are potted early in the spring and given heavy application of fertilizer rich in nitrogen and phosphorus (16-20-0 Ammophos in these tests). Before the potted plants produce runners, special propagating flats are prepared. Deep flats with 1/6-in. mesh hardware-cloth bottoms are filled with a mixture of equal parts washed mason's sand, sawdust, and sandy loam soil and are steam

[6] Stakman, E. C., M. N. Levine, and W. Q. Loegering. 1944. Identification of physiologic races of Puccinia graminis tritici. U. S. Dept. Agr., Bur. Ent. and Pl. Quar. E-617, 27 p.

[7] Stakman, E. C., M. N. Levine, and D. L. Bailey. 1923. Biologic forms of Puccinia graminis on Avena spp. Jour. Agr. Res. 24: 1013-1018.

[8] Shu, P., Kathleen G. Tanner, and G. A. Ledingham. 1954. Studies on the respiration of resting and germinating uredospores of wheat stem rust. Canad. Jour. Bot. 32: 16-23.

[9] Hotson, H. H., and V. M. Cutter, Jr. 1951. The isolation and culture of Gymnosporangium juniperi-virginiae Schn. upon artificial media. Natl. Acad. Sci. Proc. 37: 400-403.

[1] Plant Pathologist, Oregon Agricultural Experiment Station, Department of Botany and Plant Pathology, Oregon State College, Corvallis, Oregon.

FIG. 1. Arrangement of potted strawberry mother plant and flat containing sterilized soil for production of runner plants free of the red-stele fungus. Photograph by H. H. Millsap.

sterilized at 12 lb. pressure for 4 hours. This mixture permits adequate drainage, and the runner plants produce luxuriant root systems in the loose sandy soil. The autoclaved soil should be kept moist after autoclaving, otherwise it becomes almost unwettable. The flats of sterilized soil should be placed on wooden frames or on sterilized pumice blocks so that they will be several inches above the soil in the pots. They should not be set directly on benches or on the soil.

As soon as runners appear, they should be carried upward and tied to bamboo stakes or wire so that they do not come into contact with the soil in which the mother plant is growing. Glass wool placed around the plant will prevent splashing of soil particles or of oospores or zoospores of the fungus onto the runners or into the propagating flats. When the runners are long enough to reach the soil in the flats, they are pegged down with wire staples or small stakes until the first runner plants are rooted.

As soon as the second runner plant on each runner is rooted, the runner can be cut loose from the mother plant. Thus the potted plant can be removed by early August, leaving only the young plants in the raised flats. These should be kept well watered and fertilized sufficiently to assure good growth.

In western Oregon, where winters are usually mild, the flats have been left during the winter in a screenhouse where they were exposed to frequent rains and cool temperatures. Red-stele can be detected easily in plants of susceptible varieties grown under these conditions. In colder climates, where freezing temperatures would cause severe root damage, the flats would have to be kept in a greenhouse that was cold enough to cause the plants to become dormant, but not cold enough to injure the roots.

In our tests, in which susceptible varieties have been used, no effort was made to start with disease-free plants or with noninfested soil. Some of the mother plants were known to be infected, and in some cases soil known to be heavily infested with *Phytophthora fragariae* was used in the pots in which the mother plants were grown. During the spring and summer these plants grew well and produced abundant runners. In 3 seasons of testing, more than 100 flats of plants have been produced by this method. Not 1 of the runner plants has shown any evidence of red-stele.—Department of Botany and Plant Pathology, Oregon State College, Corvalis, Oregon.

Purification and Stability of the Yellow Streak Mosaic Virus of Wheat.[1] W. C. BURGER.[2] It is evident from the electron microscope studies and attempts to purify isolates of the streak mosaic viruses of wheat by Gold et al[3] and Sill[4] that these viruses occur at relatively low concentrations in host-plant juice and that the infective virus is not easily obtained in a

[1] Published with the approval of the Director as Paper No. 695, Journal Series, Nebraska Agricultural Experiment Station.

[2] Agent, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Present address: Barley and Malt Laboratory, Madison, Wisconsin.

[3] Gold, A. H., B. R. Houston, and J. W. Oswald. 1953. Electron microscopy of elongated particles associated with wheat streak mosaic. Phytopathology 43: 458-459.

[4] W. H. Sill, Jr. Private communication.

purified condition. The present work with the type culture[5] of wheat yellow streak mosaic virus indicates that the difficulties attending the purification of these viruses are not solely the result of low virus concentration in host-plant juice but also the result of marked instability of the viruses when separated from wheat plant juice.

Preparations of the yellow streak mosaic virus obtained from expressed juice of infected wheat plants by 2–4 cycles of differential centrifugation in distilled water at 3000 G for 30 minutes and 79,000 G for 1 hour were found to be noninfective. After a single cycle of centrifugation, the virus still was infective, but the infectivity decreased rapidly, and the preparation was inactive after 16 hours in distilled water at 4°C. This was in marked contrast to freshly expressed infective wheat juice, which exhibited no loss of infectivity after 6 days at 4°C and remained infectious after 14 days at this temperature. Infectivity assays were made on Michigan Amber wheat plants in the 3-leaf stage by wiping individual leaves once with the inoculum containing approximately 2 mg of 500-mesh Carborundum per ml. An average of 27 plants was used for each solution assayed. Dilutions of 1/10, 1/50, and 1/100 were used except that concentrated preparations were tested at higher dilutions.

The results indicate that the virus remains infective for only a few hours after being sedimented by centrifugation and that during the time required for further purification the virus is inactivated in the absence of materials present in the plant juice. This may explain some of the results of workers who have been unsuccessful in purifying this or similar strains of the virus by conventional differential centrifugation methods.

The removal of the majority of the cytoplasmic proteins from freshly expressed infective wheat juice by acidification did not hasten the inactivation of the virus. Protein was determined by treating portions of the solutions to be analyzed with a 10 per cent solution of trichloroacetic acid in water for 1 hour at ice-bath temperature. The resulting precipitates were washed 3 times with distilled water by centrifugation, dried *in vacuo* over P_2O_5, and weighed. Plant juice containing 16.9 mg of protein per ml and having a dilution end-point of 1/100 in distilled water was adjusted to pH 4.9 by the addition of glacial acetic acid and held for 1 hour in an ice bath. The resulting coagulum of protein was separated by centrifugation at 3000 G. The clarified supernatant, which contained 2.6 mg of protein per ml, had a dilution end-point of 1/100. Following adjustment to pH 6 with N NaOH, the supernatant was allowed to stand at 4°C for 20 hours. Only a small decrease in infectivity occurred during this time. The virus then was concentrated 130 fold by a single centrifugation at 79,000 G for 1 hour. Most of the protein in the resulting pellet was found to be insoluble in distilled water; this insoluble protein was removed by low-speed centrifugation. The resulting supernatant contained 3.6 mg of protein per ml and had a dilution end-point of 1/6500 when assayed immediately; however, this solution also lost all virus activity after 16–20 hours at 4°C. Thus it appears that infective wheat plant juice contains stabilizing materials that are not precipitated at pH 4.9 along with approximately 80 per cent of the plant juice proteins. Only after it is separated from these materials does the virus undergo rapid inactivation.

Several attempts were made to preserve infectivity of the partially purified virus by addition of substances known to act as preservatives for labile proteins. Portions of freshly purified virus were diluted with measured volumes of dilute solutions of glycerol, glucose, sucrose, and gelatin. These solutions had been previously adjusted to pH 5.5 without the use of a buffer. The mixtures were held at 4°C for 20 hours, diluted for assay, and the virus infectivity determined. Virus solutions containing 0.1 per cent gelatin retained about 80 per cent of the activity of the freshly purified material in distilled water, whereas the solutions containing 1 per cent glycerol, glucose, and sucrose retained 70, 60, and 60 per cent, respectively. Because gelatin is nonantigenic, its use in the purification and storage of the wheat streak mosaic viruses for serological purposes may prove of value.

It is evident that a major portion of the original active virus is preserved in dilute solutions of a number of polyhydric substances or gelatin. The fact that substances as dissimilar as gelatin and glycerol are effective in preserving virus activity suggests that the effectiveness of such materials, including materials present in the infective plant juice, may be related to their colligative properties, such as the ability to affect osmotic pressure. This does not appear to be true, however, for gelatin at a concentration of roughly 10^{-5} M (0.1 per cent) is more effective than are glycerol, glucose, or sucrose at concentrations of 10^{-2} to 10^{-1} M (1 per cent).

No satisfactory explanation has been given for the fact that many carbohydrates, glycerol, polyalcohols, and some proteins retard the denaturation of various labile proteins in solution[6, 7]. It appears that the process by which the rapid inactivation of the yellow streak mosaic virus may be retarded is of a similar nature. A combination of wheat plant cytoplasmic proteins and soluble carbohydrates may be responsible for the protective effect of untreated plant juice upon yellow streak mosaic virus.—Department of Plant Pathology, University of Nebraska, Lincoln 3, Nebraska.

[5] McKinney, H. H. 1944. Descriptions and revisions of several species of viruses in the genera Marmor, Fractilinea and Galla. Wash. Acad. Sci. Jour. 34: 322-329.

[6] Fraenkel-Conrat, H. 1951. Chemical reactions of proteins. In D. M. Greenberg, ed. Amino acids and proteins. Charles C. Thomas. Springfield, Ill. p. 532-585.

[7] Putnam, F. W. 1953. Protein denaturation. In H. Neurath and K. Bailey, ed. The proteins. Academic Press. New York, v. 1, p. 807-892.

Peppermint Wilt Induced by a Verticillium Isolate from Potato.—C. A. THOMAS AND R. E. WEBB. Verticillium wilt (caused by *Verticillium albo-atrum* Reinke & Berth.) is regarded as the most serious disease of peppermint (*Mentha piperita* L.) in Indiana and Michigan. A knowledge of the variations in pathogenicity and host range of isolates of the causal organism is important to the development of successful control measures. The complete specificity of Verticillium isolates from mint was reported by Nelson[1] in 1950. Verticillium isolates obtained by him from other suscepts were not pathogenic to mint. In 1951, Green[2] found that certain Verticillium isolates from mint could be recovered from pepper and eggplant 45 days after artificial inoculation. He did not state whether typical wilt symptoms were produced in any of the suscepts. Horner[3] concluded from a recent study of Verticillium isolates that peppermint can be a symptomless host for strains of Verticillium capable of causing disease in other crop plants. This note concerns a Verticillium isolate from potato capable of inducing wilt symptoms in peppermint.

Mitcham peppermint plants, grown in steamed soil from rooted cuttings, were used to determine the pathogenicity of 7 *Verticillium* isolates known to be pathogenic to potato, tomato, safflower, or mint. The first series of inoculations was made 1 week after planting the rooted cuttings in pots. Inoculation consisted of inserting fungus growth from an agar culture into a cut in the stem at the soil line. Ten plants, each in a 4-in. clay pot, were inoculated with each isolate.

The isolates that produced wilt symptoms in peppermint were used in a second series of inoculations. Rooted Mitcham peppermint cuttings, seedling Marglobe tomato plants, and young Kennebec potato plants were inoculated by dipping the root systems in a water suspension of spores and mycelium at the time of transplanting to pots of steamed soil. Sixteen plants, 4 replications of 4 plants each, were inoculated with each isolate.

Noninoculated controls were used in both series. All tests were continued for 8 weeks in a greenhouse with a mean temperature of 21°C. Reisolations were made from peppermint.

Three isolates produced typical wilt symptoms in the wound-inoculated peppermint plants. Two of these isolates were from wilted peppermint in Indiana. The third isolate, 9A, was a subculture of a pure white non-microsclerotial sector produced by isolate 9 from Katahdin potato in Maine. Other isolates from potato, tomato, or safflower did not produce wilt symptoms in peppermint.

Isolate 9A, as well as the 2 isolates from mint, produced wilt symptoms in the root-inoculated peppermint within 4 weeks. After 8 weeks, however, it was evident that the symptoms produced by 9A were milder than those produced by the mint isolates. Some of the plants inoculated with 9A showed a tendency to outgrow the wilt. Isolates 9 and 9A produced typical wilt symptoms in potato and tomato. The isolates from mint produced no visible symptoms in these 2 suscepts.

Isolate 9A was recovered from root-inoculated peppermint as much as 6 in. above the soil line. The 2 isolates from mint were reisolated as much as 10 in. above the soil line. Cultures of the reisolates resembled the original isolates in cultural characteristics. The reisolate of 9A was pathogenic to peppermint, tomato, and potato and showed no loss in virulence.

So far as is known this is the first report of a Verticillium isolate capable of inducing wilt symptoms in peppermint as well as potato or tomato. This work and previous reports[1,2,3] indicate that such strains of *V. albo-atrum* are not common. The occurrence of such strains, however, and their maintenance or build-up on other hosts as demonstrated by Horner,[3] may become important.—Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

Phloem Discoloration of Sweet Orange.[1] FARID NOUR-ELDIN. During a survey in November, 1954, in the Dakhla and Kharga oases in Egypt, most balady (meaning common or local) sweet orange trees (*Citrus sinensis* Osbeck) budded on sour orange rootstock exhibited phloem discoloration. In surveys of the citrus diseases of Egypt[2], phloem discoloration was found on the following sweet orange varieties: Balady, Navel, Valencia, Sukkary, Khalily White, Balady Blood, and Sanguinoval. More than half of the observed cases were present in the Balady variety, and the rest were scattered more or less equally among the other varieties on trees of not less than 5 years of age.

Affected trees usually are stunted. When the cortex at the bud union of affected trees is carefully cut away, the phloem of the sweet orange scion is found to be discolored (Fig. 1, A). The discoloration usually is limited to an area of an inch or less above the bud union, but sometimes it extends 24 in. above the bud union. The phloem of the sour orange rootstock has never shown any indication of discoloration. Phloem discoloration has been recorded on sweet orange

[1] Nelson, R. 1950. Verticillium wilt of peppermint. Mich. Agr. Expt. Sta. Tech. Bul. 221, 259 p.

[2] Green, R. J. 1951. Studies on the host range of the Verticillium that causes wilt of *Mentha piperita* L. Science 113: 207-208.

[3] Horner, C. E. 1954. Pathogenicity of Verticillium isolates to peppermint. Phytopathology 44: 239-242.

[1] The author wishes to acknowledge his indebtedness to Dr. J. F. L. Childs, Citrus Experiment Station, Orlando, Florida, for reading the manuscript and for his valuable suggestions; to Dr. H. Schneider, Citrus Experiment Station, Riverside, California, for examining the specimens sent to him and for his helpful suggestions; and to A. Omar and K. Jacob for their help during the survey.

[2] Childs, J. F. L., F. Nour-Eldin, K. Jacob, and N. El-Hosseiny. 1955. Unpublished report to the Egyptian Ministry of Agriculture on the survey of citrus diseases in Egypt. February-July, 1955.

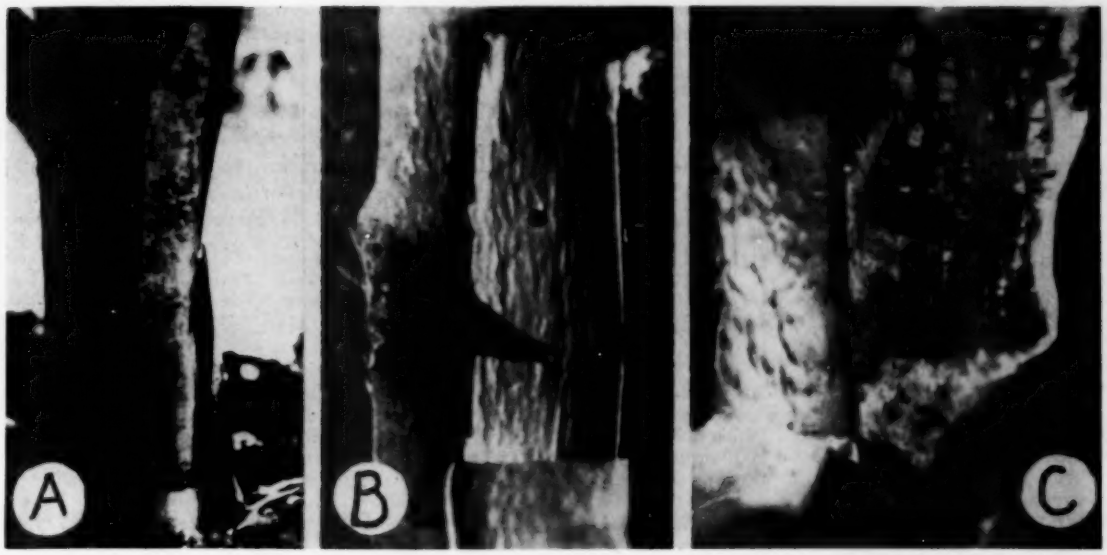

FIG. 1. A) Phloem discoloration of sweet orange (Balady variety) scion on sour orange rootstock. Note the absence of phloem discoloration of the rootstock. B) channeled pitting on the wood of sweet orange (Balady variety) scion. C) Cachexia-like symptoms of Balady variety of mandarin. Note phloem discoloration and stem pitting of the mandarin scion and its absence on the sour orange rootstock.

varieties in China[3]. An extremely mild phloem discoloration in Florida on Pineapple and Valencia sweet orange varieties on rough lemon stock was reported to the author by J. F. L. Childs.

In addition to the phloem discoloration, pitting of the sweet orange wood was sometimes observed. In such cases, the inner face of the bark above the bud union was marked with projections that coincide with and fit into pits in the cambial face of the wood.

Two types of pitting have been observed. One gives a grooved or channeled appearance to the cambial face of the wood (Fig. 1, B), and the other is a round or conoid type of pitting. Discoloration of the pegs has not been observed. Phloem discoloration of affected trees was not necessarily accompanied by stem pitting. Few trees with stem pitting, however, were free of phloem discoloration. In a few instances, the sour orange stock exhibited very slight stem pitting when pitting occurred on the scion.

The phloem discoloration and stem pitting symptoms seen in Egypt resemble the symptoms of the cachexia disease of Orlando tangelo (*C. paradisi* × *C. reticulata*).[4,5] The casual agent of cachexia has been transmitted through buds,[4,5,6] but 2 sweet orange varieties infected with the causal agent of cachexia disease showed no phloem discoloration in Florida.[5] In the survey in Egypt, typical cachexia-like symptoms were observed on balady mandarin trees (*C. reticulata*) budded on sour orange (Fig. 1, C). It should be noted that the Balady variety of sweet orange can be considered as a very early Valencia variety.

Until experiments on transmission have been completed, it is premature to discuss the relationship of this disease with other citrus diseases that produce phloem discoloration and stem pitting symptoms. The general appearance and orchard distribution of the trees with phloem discoloration, however, suggest that the causal agent of the disease is bud-transmitted from infected mother trees.—Plant Pathology Section, Ministry of Agriculture, P. O. Orman, Egypt.

[3] Kung-Hsiang, L. 1943. Convex gum, a new disease of citrus in China. Phytopathology 33: 394-397.

[4] Childs, J. F. L. 1950. The cachexia disease of Orlando tangelo. U. S. Dept. Agr. Pl. Dis. Rptr. 34: 295-298.

[5] Childs, J. F. L. 1952. Cachexia disease, its bud transmission and relation to xyloporosis and tristeza. Phytopathology 42: 265-268.

[6] Olson, E. O. 1954. Some bark and bud union disorders of mandarin and mandarin-hybrid rootstocks in Texas citrus plantings. Amer. Soc. Hort. Sci. Proc. 63: 131-136.

REPORT AND ABSTRACTS OF THE 1955-56 WINTER MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The eleventh annual meeting of the Northeastern Division of the American Phytopathological Society was held at West Springfield, Massachusetts, on November 3 and 4, 1955. Seventeen scientific papers

were presented. The evening meeting was devoted to a discussion on breeding for disease resistance. Dr. George B. Reynard discussed breeding for disease resistance in vegetable crops, and Dr. L. Frederic Hough gave a progress report on the cooperative program being conducted on breeding of apples for scab resistance. Approximately 125 people attended.

The matter of a summer meeting was left in the hands of the executive committee. The invitation of the Eastern States Farmers' Exchange to use their facilities at West Springfield for the next annual meeting was accepted, with November 8 and 9 as the tentative dates.

Officers elected for 1956 were F. O. Holmes, *President;* B. H. Davis, *Vice President;* and B. A. Friedman, *Secretary-Treasurer.* E. G. Rex was elected in 1954 to serve as councilor for a two-year term.

B. H. Davis, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Streptomycin for control of blue mold and bed rot of tobacco. Anderson, P. J. On replicated, inoculated plots in tobacco beds, both streptomycin and Dithane Z-78 (65 per cent zineb) sprays gave good control of blue mold (caused by *Peronospora tabacina*). It made no difference whether the streptomycin was applied once or twice a week, or whether the concentration was 200 ppm or 400 ppm. Bed rot (caused by *Pythium aphanidermatum*) was controlled perfectly by Dithane Z-78 (2 lb. to 50 gal. of water, twice a week), but streptomycin was of no value against this disease. After the spray program was stopped, blue mold appeared in less than a week on all plots, regardless of the spray material used. Thus, the protection of the plants does not last longer with streptomycin than with Dithane Z-78. The results suggest that both of these materials are fungistatic rather than fungicidal.

Effect of nutrition on the incidence of bacterial spot of peach. Bachelder, Stephen, Robert H. Daines, and Clayton E. Bartley. In the early spring of 1949, 1950, and 1951, fertilizer materials were applied beneath 4- to 11-year-old trees of Jerseyland, Sunhigh, and Afterglow peach varieties in 21 orchards in southern New Jersey. These materials supplemented standard fertilizer applications of growers. Annual rates per acre for each of 4 treatments were 500 lb. ammonium sulfate, 1000 lb. super phosphate, 400 lb. muriate of potash, 15 lb. minor element mixture containing Borax, 30 lb. zinc sulfate, 30 lb. copper sulfate, 180 lb. magnesium sulfate, 100 lb. ferrous sulfate, 50 lb. manganese sulfate, and 1 lb. sodium molybdate. In each orchard, 5–7 randomized replicates were used for each treatment and check. Natural infections by *Xanthomonas pruni* (E. F. Sm.) Dowson occurred each year. At each annual harvest, 800 or more peaches from treated and check trees were examined. For each of the 3 years, the amount of bacterial spot from high-phosphorus treated trees was significantly less than that from high-nitrogen trees. For 2 years, fruit from high-nitrogen trees had significantly more bacterial spot than did fruit from potassium-treated and check trees. Excessive nitrogen appears to increase slightly the susceptibility of peach fruit to bacterial spot. It is doubtful that fertilization offers a practical means of control.

Effect of viruses on plant production and yield of strawberries in New Hampshire. Becker, R. F., and A. E. Rich. During the 1954 and 1955 growing seasons, a comparison was made between virus-free and locally grown Catskill, Premier, and Sparkle strawberry plants with reference to plant production and fruit yield. The local plants were obtained from growers who maintained their own stock, and the virus-free plants were supplied by the U.S.D.A. at Beltsville, Maryland. Ten virus-free and 10 local plants of each variety were set in May, 1954, in newly cleared land, using a 5×7-ft. spacing. During the summer, all runner plants were spaced as evenly as possible, and counts were made periodically during the season. In a nematode survey, parasitic species were recovered from 3 clones (2 Catskill, 1 Premier) of the local plants. Because of severe symptoms and low yields, no data from these clones or their paired virus-free counterparts were included. The average numbers of runners per mother plant on October 4 for the virus-free Catskill, Premier, and Sparkle were 89, 160, and 95, respectively, and for the local plants, 39, 93, and 81, respectively. Yield data were taken in 1955. The average yield per clone for the virus-free Catskill, Premier, and Sparkle was 10.6, 13.5 and 10.9 quarts, respectively, as compared to 3.9, 8.0, and 7.2 quarts, respectively, for the local plants.

The effect of various soil amendments on maple wilt incited by Verticillium sp. Caroselli, N. E. Certain soil amendments, applied either 1 month or 9 months prior to the inoculation of well-established field-grown sugar maple trees, appeared to reduce or increase the degree of wilt severity. Randomized trees, ranging from 1¾ to 6 in. diameter at breast height (dbh) and inoculated with a virulent isolate (V-10) of *Verticillium* sp., were separately treated with ammonium sulfate, sodium nitrate, NuGreen, Milorganite, Cyanamid, and a 6–8–7 fertilizer. Compounds were applied to the soil surface at the rate of ⅛, ¼, and ½ lb. of nitrogen for every in. dbh. At least 5 replicates were used for each treatment. Some degree of control was obtained by all treatments except sodium nitrate. Ammonium sulfate, regardless of the time of treatment, afforded the best control, which was consistent from the onset of symptoms to the end of the season. Trees treated with sodium nitrate, regardless of the amounts used, exhibited more severe wilt symptoms than those of the check trees. In further tests where small trees were treated with ammonium sulfate and with sodium nitrate, the mean wilt at the end of the season was 20 per cent and 39 per cent, respectively, compared with 35 per cent for the untreated trees.

The fungicidal activity of hydrocarbons as influenced by structure, aeration, and irradiation. Daines, Robert H., Eileen Brennan, and Ida Leone. The fungicidal activity of simple hydrocarbons, as determined by standard bioassay techniques, has some relationship to their chemical activity. Among the open-chain compounds, those possessing olefinic groups were shown to have more activity than saturated ones. In general, the straight-chain primary olefins having approximately 8–10 carbons were most effective. The cycloparaffins also possessed fungicidal activity that was enhanced by an olefinic group. Since olefins are converted to peroxides on contact with air, a study was conducted to determine the effect of aeration and irradiation on these hydrocarbons. The compounds studies showed increasing activity as the treatment time was extended. Of the hydrocarbons treated, those possessing tertiary hydrogens showed greatest activity increase. Aromatic compounds with relatively short-chain alkyl groups possessed fungicidal activity. Such compounds possessing a tertiary hydrogen that can be oxidized to a hydroperoxide, also showed improved activity on aeration and irradiation. Partially hydrogenated aromatics having condensed nuclei

showed relatively good activity as fungicides. Although the cause of this activity is unknown, such compounds may act as hydrogen donors.

Behavior of juice extracts of certain virus isolates from prunus and from rose in cucumber plants. GILMER, R. M. The behavior of 123 virus isolates from several *Prunus* species and of 2 virus isolates from *Rosa multiflora* was studied in cucumber plants. The isolates from Prunus were obtained from 9 states and from Canada. All isolates were initially transmitted to Elberta peach and to *Prunus tomentosa* by budding. Mechanical transmissions from peach leaves to cucumbers occurred readily, but no transmissions to cucumbers from *P. tomentosa* were accomplished. Syndromes incited in cucumber by all 125 isolates were similar. The effects of pH, of aging of juice extracts, and of certain cations and anions on infectivity were determined for 20 representative isolates. All isolates were infective in juice extracts buffered in the pH range 4.2–11.6, but all were irreversibly inactivated at pH 3.8 and 12.0. The effects of heavy metal cations and of certain anions on infectivity of each isolate were similar. Thermal inactivation points were almost identical. They increased as the season progressed. Some differences in the effects of aging on infectivity of the individual isolates were noted. The data suggest that the syndrome incited in cucumber is due to a single virus entity and that this entity is probably necrotic ring spot virus.

Datura spp. as indicator plants for apple and blueberry virus diseases. HILBORN, M. T., AND REINER BONDE. A disease of apple, affecting particularly the variety Virginia Crab, has been suspected by other investigators to be caused by a virus. The symptoms on Virginia Crab resemble tristeza disease of citrus. Inoculations were made by stripping the bark from succulent 1-year-old shoots of apple and gently rubbing the moist inner bark-cambial layer so exposed on the leaves of *Datura innoxia.* Leaf necrosis occurred on the Datura when use was made of 1) Virginia Crab wood, 2) a root sucker from the seedling root on which the Virginia Crab was propagated, and 3) McIntosh and Baldwin wood from trees top-worked on Virginia Crab. No symptoms on Datura were produced when McIntosh and Baldwin trees not top-worked on Virginia were used. It has been suspected that some of the leaf spots found on wild low-bush blueberry plants were caused by viruses. Attempts to transmit a virus by grafting or by juice inoculations of several commonly used indicator plants failed. In 1955, 3 types of symptoms were produced on *Datura stramonium* by dodder connections from spotted blueberry plants. Inoculations from a yellowish leaf spot of blueberry caused severe leaf blotching involving the entire tip, those from plants with a brown leaf spot caused circular necrotic spots, and those from blueberry plants with dark ring-like spots caused a necrosis along the midvein.

The mode of infection of low-bush blueberry by Exobasidium vaccinii. HILBORN, M. T., AND FAY HYLAND. It has long been known that the red leaf disease (caused by *Exobasidium vaccinii*) is systemic in low-bush blueberry (*Vaccinium* spp.). The mycelium is perennial in the rhizome, and new shoots arising from an infected rhizome may show symptoms of the disease during their first year of growth. Histological examination of infected plants showed that the mycelium was rather generally distributed throughout the rhizome and was present in the shoot buds arising along the rhizome, even before they developed into new shoots. In a 1-year-old shoot, the mycelium was widespread in parenchyma cells. It was most conspicuous in the pericycle. The fungus fruits in June and early July on the lower surfaces of leaves, and spores may alight along the stem and cause infection by direct penetration through the cuticle. Once in the cuticle, the mycelium spreads netlike throughout the cortex and may penetrate from there to the pith. This type of infection has been observed only on stems, and it is not known if leaf infection can also occur. This mode of infection may explain the rapid spread of the red leaf disease throughout blueberry fields that previously showed only a trace of the disease.

Nontoxicity to elms, oaks, and maples of 1,2-dibromo-3-chloropropane applied as a soil drench. HOLMES, F. W. 1,2-Dibromo-3-chloropropane, reported to prevent nematode injury to citrus and peach trees, was applied to the soil around 3 elms, 3 maples, and 2 oaks at each of 3 concentrations: 2.5, 5, and 10 gal. per acre. This compound, combined with Stoddard Solvent and Emcol H85 in the proportion of 27.9:66.3:5.8, was pumped around trees between dikes. The mixture was applied as a soil drench in 5 in. of water on June 16, several days after the soil temperature at a 6-in. depth had reached 65°F. No phytotoxic symptoms or other differences between treated and untreated trees of each species were noted during the summer or fall. Growth of rye, however, was strongly retarded in adjacent portions of an adjoining field on the downhill side.

Nuclear number in conidia of Verticillium spp. KHOO, UHENG, H. T. STINSON, AND PAUL E. WAGGONER. Variation has been observed within species of *Verticillium.* The separation of the 2 species *Verticillium albo-atrum* and *V. dahliae* has not been fully accepted because of the variation in production of microsclerotia within cultures. Furthermore, isolates from potato vary in their pathogenicity to Kennebec potatoes. The number of nuclei in a conidium determines whether a single-spore culture starts with a homogeneous nuclear genetic complement. Conidia of nonmicrosclerotial cultures from potato and from microsclerotial and nonmicrosclerotial sectors of a sectoring culture from cotton were stained according to DeLamater's method. Conidia were fixed in osmic acid vapor, hydrolyzed in HCl, and stained in thionine-SO_2. A total of 1339 conidia were examined. Of these, 1334 were uninucleate and 5 possibly binucleate. Anastomoses were seen between germ tubes from different conidia.

Two distinct types of corn stunt disease in Mexico. MARAMOROSCH, KARL. A field survey in Mexico revealed the presence of 2 distinct types of corn stunt disease. The type designated as Rio Grande corresponds to the stunt previously reported from California and Texas. It was found for the first time in Mexico, in the south (Oaxaca), north (Ciudad Victoria), and east (Vera Cruz) of the country. The type designated as Mesa Central, known previously from the Central Plateau and Gulf Coast regions, was also found on the Pacific Coast. The characteristic features of the Rio Grande type are interrupted chlorotic streaks on leaves and numerous secondary shoots. In the Mesa Central type, the chlorotic spots are absent or are less conspicuous than in the Rio Grande type. The Mesa Central disease causes red to purple discoloration of the leaves and more branching at the base and more stunting than does the other type. Several commercial U. S. varieties of corn were inoculated with each type of the causative viruses by means of the vector *Dalbulus maidis.* The symptoms of each type were consistently reproduced irrespective of the variety used. *D. maidis* proved an efficient vector for both types. Whether the 2 types of corn stunt are caused by strains of the same virus or by 2 unrelated viruses has not yet been established.

The association of Rhizoctonia solani and nematodes in a root rot of winter wheat. MOUNTAIN, W. B., AND W. G. BENEDICT. The fungus *Rhizoctonia solani* and a root-lesion nematode, *Pratylenchus minyus,* were found to be closely and consistently associated with a root rot of

winter wheat. Symptoms included stunting and occasional killing of the plant, reduced tillering, yellowed leaves, purple leaf sheaths, and a greatly reduced root system with extensive cortical lesions. In greenhouse and field experiments where the effects of the 2 pathogens upon plant growth were separated by differential chemical treatments of the soil, the combined effect of the fungus and the nematode upon growth of the wheat was almost twice as great as the effect produced by either pathogen alone. As the incidence of fungus appeared to be correlated with the endoparasitic nematode population, it is possible that there is an interrelation of the 2 organisms in the infection court, although direct proof could not be obtained for this.

Influence of insecticide and fungicide sprays on downy mildew of broccoli. NATTI, JOHN J., AND G. E. R. HERVEY. For the past 3 years, downy mildew (caused by *Peronospora parasitica*) has been observed to be more prevalent in plantings of broccoli that had been sprayed with insecticides than in unsprayed plantings. In 1955, broccoli plants that had been sprayed with an emulsifiable insecticide formulation averaged 30 mildew lesions per 9 sq. in. of leaf area, whereas unsprayed plants and plants sprayed with an experimental phosphate compound containing no solvent averaged 20 lesions. The increase in incidence of mildew in plots sprayed with the emulsifiable insecticide formulation appeared to be related to the dissolving of the bloom and of wax in the cuticle of the broccoli leaves by the solvent contained in the emulsions. Spergon SL (95 per cent chloranil) at 3 lbs. per acre and Agri-mycin 100 (15 per cent streptomycin and 1.5 per cent oxytetracycline) at 1 lb. per acre were the most promising materials in tests on control of down mildew by foliar sprays applied at both low- and high-gallonage rates. Captan 90W (90 per cent captan) at 3.3 lb. per acre, Dithane D-14 (14 per cent nabam) at 2 quarts plus zinc sulfate at ¾ lb., and B 622 (50 per cent 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine) at 6.6 lb. were not effective. Control of downy mildew in these tests with Agri-mycin and the reported control of blue mold of tobacco (caused by *P. tabacina*) with streptomycin suggests that streptomycin is effective against the Peronosporaceae.

Can fungi develop tolerance to fungicides? PARTRIDGE, A. D., AND A. E. RICH. In an attempt to determine whether or not fungi are capable of developing a tolerance to fungicides, *Penicillium notatum*, *Scelerotinia fructicola*, and *Stemphylium sarcinaeforme* were grown on potato-dextrose agar containing various concentrations of copper sulfate, mercuric chloride, phenyl mercuric acetate, glyodin, and captan. Periodic transfers were made on media containing the highest concentrations of fungicides at which good growth would occur. At intervals the fungi were transferred to media containing concentrations in excess of the original maximums. *P. notatum* showed an increase in tolerance to copper sulfate, mercuric chloride, and captan but not to phenyl mercuric acetate or glyodin. *Sc. fructicola* and *St. sarcinaeforme* showed an increase in tolerance to copper sulfate, mercuric chloride, glyodin, and captan but not to phenyl mercuric acetate. All organisms retained their tolerance to copper sulfate through a number of transfers on media containing no fungicides, but some loss of tolerance was noted when organisms grown in the presence of mercuric chloride, glyodin, or captan were carried through a number of transfers on media containing no fungicides.

Field control of root knot nematode in onion muck by the use of fumigants. SHERF, ARDEN F., AND K. W. STONE. Duplicate 1/10-acre plots of muck heavily infested with root nematodes were fumigated with D-D (1,3-dichloropropene; 1,2-dichloropropane) at 40 gal./acre, Dowfume W-85 (1,2-dibromoethane) at 6 gal./acre, and N-869 (*N*-methyl dithiocarbamate dihydrate) at 25 gal./acre by means of tractor-mounted commercial injection equipment. Outstanding differences in leaf color, rate of bulbing, and yield of bulbs were noted. These influences were correlated with the degree of nematode infestation. Total yields in lb. per acre were 37,030 for untreated, 53,090 for D-D, 49,636 for W-85, and 42,242 for N-869. W-85 caused a moderate inhibitory effect on onions, expressed as a delay of growth and of maturity with improper sizing of bulbs. Observation of carrots, lettuce, and radish that were spot planted in the plots indicated successful nematode control with D-D and W-85 and poor control with N-869. Although onion (*Allium cepae*) is classed as resistant or tolerant to *Meloidogyne* spp., this work indicates that in certain New York muck soils the crop is definitely susceptible.

Glycerol as an adjuvant in a streptomycin sulfate spray for field control of halo blight of Red Kidney bean. SILBER, GUSTAVE, AND JOHN KAINSKI. A spray of 300 ppm streptomycin sulfate (STS formulation) was compared with one of 200 ppm streptomycin sulfate plus 0.5 per cent glycerol for the control of halo blight (caused by *Pseudomonas phaseolicola*) of Red Kidney bean in field plots planted with a 1:5 mixture of infected and healthy bean seed. Weekly sprays on 8 replicated plots per treatment were begun as the first trifoliate leaves were expanding. Five days after the tenth spray, 100 leaves were collected randomly from each sprayed plot and indexed for disease severity as follows: 1) 0 lesions, 2) 1–2 lesions, 3) 3–10 lesions, 4) 11–25 lesions, and 5) over 25 lesions. Of the leaves sprayed with 300 ppm streptomycin sulfate, 39, 20, 19, 12, and 10 per cent were indexed into class 1, 2, 3, 4, and 5, respectively. The corresponding percentages for leaves sprayed with 200 ppm streptomycin sulfate plus glycerol were 28, 17, 25, 13, and 17. Sprayed plants were vastly superior to unsprayed ones; those sprayed with 300 ppm streptomycin sulfate were superior (odds 99:1) to those sprayed with 200 ppm streptomycin sulfate plus 0.5 per cent glycerol. The data suggests that when inoculum levels are high, 0.5 per cent glycerol in a streptomycin sulfate spray will not substitute for higher concentrations of actual toxicant in field usage.

Tobacco seedbed treatments for control of weeds and soil-borne pathogens. TAYLOR, GORDON S. New, easily applied compounds were compared with steam, methyl bromide, and chloropicrin as herbicide-fungicides in tobacco seedbeds. Seedbeds, worked 6 in. deep after adding 1 in. of swamp humus, were sowed in October with weed seed. Treatments were applied next day (soil temperature 56°F) to plots of 54 sq. ft. each, replicated 3 times. Treatments and rates of application per plot were the following: steam, for 30 minutes; methyl bromide, 1 lb.; Vapam (sodium methyldithiocarbamate), 0.5 lb. in 8 gal. water plus 8 gal. water seal; allyl alcohol, 260 ml in 6 gal. water plus seal; chloropicrin, 245 cc (injected); and check. In May, weed counts per sq. ft. were 2, 2, 27, 97, 126, and 141, respectively. Differences were highly significant statistically. Beds then were weeded, and mean dry weight of 50 tobacco plants was determined in mid-June. These weights in g were 5.4, 5.8, 6.7, 3.6, 6.8, and 3.8, respectively. Differences were statistically significant. A Pythiacious fungus with small oospores was abundant in the roots of the stunted plants in the check and allyl alcohol plots. Chloropicrin and allyl alcohol did not control weeds. Allyl alcohol did not control soil-borne pathogens. Vapam appears promising as an easily applied, fungicidal-herbicidal treatment for tobacco seedbeds. Safe dose range and cumulative effects need further study.